ENDOCRINOLOGY

Published Monthly, for The Association for the Study of Internal Secretions

Volume 29 AUGUST, 1941 Number 2

CONTENTS

Does Pregnancy Suppress the Lactogenic Hormone of the Pituitary. *C. W. Turner and Joseph Meites* 165

Variations in the Yield of Gonadotropic Material from Green Plants in Relation to the Season of Growth and the pH of the Fresh Juice. *Maurice H. Friedman and John W. Mitchell* 172

The Site of Elaboration of the Pituitary Gonadotropic Hormone and of Prolactin. *Maurice H. Friedman and S. R. Hall* 179

Effect of Light on Sexual Maturation, Estrous Cycles, and Anterior Pituitary of the Rat. *Virginia Mayo Fiske* 187

The Structure of Bowman's Capsule in Castrate and Testosterone Treated Male Mice as an Index of Hormonal Effects on the Renal Cortex. *Charlotte E. Crabtree* 197

Influence of Pregnancy on the Renal Cortex of Mice as Indicated by the Structure of Bowman's Capsule. *Charlotte E. Crabtree* 204

Progesterone-like Effect of Ascorbic Acid (Vitamin C) on the Endometrium. *S. Leon Israel and David R. Meranze* 210

Effect of Estrogenic Substance on Experimentally Hyperthyroid Male Rats. *T. C. Sherwood* 215

Relation of the Changes in Testicular Structure Induced in the Rabbit by Estrogenic Substance to Resistance against Syphilis. *C. N. Frazier, C. K. Hu and W. C. Ma.* 218

Influence of Estrone on the Electric Characteristics and the Motility of Uterine Muscle. *Emil Bozler* 225

Hormonal Content of the Pharyngeal Pituitary Gland. *Stephen Tolins and Robert A. Moore.* 228

Chronic Toxicity Studies of Diethylstilbestrol. II. Subcutaneous Implantation of Pellets in Rats. *R. C. Page, H. K. Russell, E. L. Schwabe, C. S. Matthews and F. E. Emery* 230

Incidence of Homoplastic Adrenocortical Transplants in Gonadectomized Rats. *Leland C. Wyman and Caroline Tum Suden* 240

The Effect of Renin on the Uric Acid Metabolism of the Pregnant and Non-Pregnant Dalmatian Dog. *N. K. Schaffer, L. V. Dill and H. J. Stander* 243

Influence of Hormones upon Phosphatase Content of Rat Femurs. I. Effects of Adrenal Cortical Substances and Parathyroid Extract. *H. L. Williams and E. M. Watson* 250

Influence of Hormones upon Phosphatase Content of Rat Femurs. II. Effects of Sex Hormones, Thyroxin and Thymus Extract. *H. L. Williams and E. M. Watson* 258

Release of Glutathione Inhibition of Melanin Formation by Estrone. *Frank H. J. Figge and Edgar Allen* 262

The Reproductive Organs in Malnutrition. Effects of Chorionic Gonadotropin upon Atrophic Genitalia of Underfed Male Rats. *Michael G. Mulinos and Leo Pomerantz* 267

Effects of Human Pregnancy Serum on Parturition in the Rat. *Samuel S. Rosenfeld, Bernard Lapan, and Harry Baron* 276

The Question of an Aqueous Hormone from the Testicle. *David Rubin* 281

NOTES AND COMMENTS

Will Anterior Pituitary-Like Substance Prevent the Degenerative Changes in the Testis Incident to Cryptorchidism? *Charles E. Rea* 288

Inactivation of Estrogens by the Liver of Guinea Pigs in Different Sexual States. *Paul Engel* 290

Annual Subscription, $10.50 *Single Copy, $1.25*

ANTERON

A MOST ADVANCED APPROACH TO THE PROBLEM OF ENDOCRINE STERILITY

ANTERON is the hormone of pregnant mare serum (PMS),—a pituitary-like gonadotropic hormone capable of stimulating the ovarian follicle and the spermatogenic tubules of the testis. Since ova and sperm are *sine qua non* for conception, the administration of ANTERON* for its potent gonadotropic stimulation may be most effective therapy in oligospermia and anovulatory sterility.

*Trade-Mark Reg. U.S. Pat. Off.

SCHERING CORPORATION · BLOOMFIELD · NEW JERSEY

ENDOCRINOLOGY

PUBLISHED FOR

THE ASSOCIATION FOR THE STUDY OF INTERNAL SECRETIONS

VOLUME 29 AUGUST, 1941 NUMBER 2

DOES PREGNANCY SUPPRESS THE LACTOGENIC HORMONE OF THE PITUITARY[1,2]

C. W. TURNER AND JOSEPH MEITES

From the Department of Dairy Husbandry, University of Missouri

COLUMBIA, MISSOURI

A NUMBER OF THEORIES, supported by experimental evidence, have been formulated to explain the absence of lactation during pregnancy and its initiation after parturition. To date none of these theories have proved to be conclusive. The cause of the initiation of milk secretion after parturition still remains unsolved.

Hildebrandt (1) was the first to suggest that the onset of lactation might be due to the removal of some suppressing agent in the blood. Halban (2), Frankl (3), Smith and Smith (4) and Nelson (5) have presented evidence indicating that the presence of the placenta during pregnancy may inhibt lactation. It was observed that when placental tissue was retained after parturition or, in one case (3) implanted near term, no lactation occurred. However, Litt (6) was unable to inhibit parturition and normal lactation by successfully implanting placental grafts into the eyes of rabbits. The placental inhibition hypothesis is further complicated by findings on the rat and mouse (7a, 7b, 8). They found that a transient lactation can occur in these animals even if they are hypophysectomized during pregnancy. This suggested to the former workers that the uterus and uterine contents might actually provide a stimulus for lactation. In this connection the results reported by Ehrhardt (9) and Lessmann (10) are very interesting. Both of these workers claim to have extracted large amounts of lactogenic hormone from placental tissue.

Selye, Collip and Thomson (11) suggested that the suppression of lactation during pregnancy may be mechanical in nature. They found that milk secretion failed to occur in rats when the young were removed by Caesarian section and the uterus was immediately distended with paraffin.

The work of Hammond (12) and Drummond-Robinson and Asdell (13) led them to conclude that the corpus luteum of pregnancy was the factor inhibiting lactation. The latter showed that if the mammary glands had reached full development in the pregnant goat, ablation of the corpora lutea resulted in immediate and abundant lactation. Selye, Collip and Thomson (14) found that when they removed the intensively luteinized ovaries of APL treated rats which had developed complete mammary glands, copious milk secretion set in

Received for publication May 2, 1941.

[1] Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station. Journal Series No. 744.

[2] This study has been aided in part by a grant from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

within 36 hours. In contrast Anselmino and Hoffmann (15) using a purified preparation of progestin and Folley and Kon (17), using crystalline progesterone, were unable to suppress established lactation in the rat by administering large quantities of these hormones.

Large doses of estrogen have been reported to inhibit established lactation, at least temporarily, in a number of animals: the mouse (18, 19, 20), the rat (16, 21, 22), the goat (23); and the cow (24, 25). The injection of estrogen in large doses followed by a sudden diminution or withdrawal was stated to elicit milk secretion in the guinea pig (26–30). On the other hand Turner and Gomez (31) found that such treatment applied to guinea pigs with well developed mammary glands failed to initiate milk secretion at all comparable to that secured by the injection of lactogen.

Nelson (30) suggested that the inhibition of lactation during pregnancy was due to estrogen acting through two channels (*a*) by suppressing the secretion of lactogenic hormone by the pituitary and (*b*) by a direct inhibitory effect on the mammary glands. The decrease in estrogen which occurs at parturition was then supposed to permit the release of lactogen from the pituitary and the consequent onset of lactation. However, this theory fails to explain the occurrence of simultaneous lactation and pregnancy in such species as the mouse, rat, rabbit, human being, goat and cow. Nor does it explain the findings (16) that it is experimentally possible to augment the lactogen content of the pituitary by the injection of estrogen into female rats and male guinea pigs. More recently it has been shown (32, 33) that the administration of limited amounts of the synthetic estrogen, stilbestrol, can actually initiate and maintain lactation for a period in virgin female goats. To harmonize these observations Folley (34) has suggested that large amounts of estrogen inhibit but small amounts stimulate milk secretion.

The discovery of the lactogenic hormone by Stricker and Grueter (35) makes it essential to consider this factor in the initiation of lactation. Certainly no realistic explanation of the initiation of milk secretion is possible without taking into account the role of the lactogenic hormone. One of the chief objections to most of the inhibition theories is the failure to consider the activity of this hormone during pregnancy and lactation. Quantitative assays of the lactogen content in the pituitaries of cows, rats and guinea pigs (16) and the pituitaries of rabbits and guinea pigs (36) have revealed the very significant fact that this hormone remains low during pregnancy but increases 2-to4-fold shortly after parturition. After parturition in women greatly increased amounts of lactogen have been found in the urine (37, 38). These data are taken to mean that lactation does not occur during pregnancy principally because the amount of lactogenic hormone produced during pregnancy is too low to support lactation.

It seems reasonable to assume that if there were any factors operating during pregnancy which inhibited lactation, they should at the same time suppress the elaboration of the lactogenic hormone by the anterior pituitary. It was the object of this experiment to determine whether or not there was any actual suppression of the lactogenic hormone during pregnancy. Answers were sought to the following questions: (*a*) What are the effects of simultaneous lactation and pregnancy on the lactogen content of the pituitary? Does pregnancy during lactation inhibit the production of lactogen by the pituitary? (*b*) Do the ovaries inhibit the lactogenic hormone of the pituitary during pregnancy? Will removal of the ovaries in advanced pregnancy result in an increase of lactogenic hormone and the initiation of lactation?

EXPERIMENTAL

Four groups of mature female New Zealand White rabbits were used in these experiments. The first group consisted of 7 does which were killed on the 20th day of pregnancy. The second group consisted of 10 lactating does which were killed on the 20th day postpartum. The third group consisted of 10 does which were rebred on the day of parturition and killed on the 20th day of simultaneous lactation and pregnancy. The litters of these last 2 groups were reduced to 2 in every case on the

5th day postpartum. It has been shown (39) that reducing the rabbit litter size to 2 has no effect on the lactogen content of the pituitary. A fourth group of animals consisted of 4 does which were ovariectomized on the 20th day of gestation; 2 of these rabbits were killed 48 hours after the operation and 2 were killed 5 days after the operation.

The animals were killed by a syringe injection of air through an ear vein. The body weight and the weights of the endocrine glands were recorded. Also the 2 young belonging to each mother in the 2nd and 3rd groups were weighed 20 days after birth. The pituitaries of each group, with the exception of the first were individually assayed for lactogen by the Reece-Turner (16) method; 10 or 20 pigeons were used for each assay. The pituitaries of the first group were combined and assayed by administering an aliquot portion to 10 pigeons. In the case of 3 of the pregnant and lactating rabbits, the placentas were extracted by the Riddle-Bates (40) method and qualitatively assayed for lactogen.

RESULTS

It would be well to mention here some of the results obtained by Holst and Turner (36) in their study of the lactogen content of pregnant and lactating rabbit

TABLE 1. LACTOGEN CONTENT OF AP OF RABBITS 20 DAYS PREGNANT

Total no. of rabbits	7
Av. body wt., gm.	3,611
Av. pituitary wt., mg.	33.43
Units per pituitary	9.75 R.T.U. 0.44 I.U.
Units per mg. pituitary tissue	0.29 R.T.U. 0.013 I.U.
Units per 100 gm. body wt.	0.27 R.T.U. 0.012 I.U.

pituitaries. They used the same breed of rabbits and assay method used in this study, and consequently their results may be compared with the data presented here. They found that does 10 days pregnant contained 11.13 Reece-Turner units (R.T.U.) of lactogen per pituitary; does 20 days pregnant contained 10.63 R.T.U. per pituitary; does 28 days pregnant contained 14.10 R.T.U. per pituitary; at 5 days postpartum the lactogen content had increased to 40.31 R.T.U. per pituitary, and at 20 days postpartum the lactogen content was 26.63 U. per pituitary.

Our results are stated in both Reece-Turner and international units of lactogen (41). It will be seen (table 1) that rabbits on the 20th day of gestation contained an average of 9.75 R.T.U. or 0.44 I.U. of lactogen. The 10 lactating rabbits (table 2) which were killed on the 20th day postpartum contained an average of 27.10 R.T.U. or 1.22 I.U. of lactogen per pituitary. The individual rabbits ranged from 22.5 to 31.25 R.T.U. of lactogen per pituitary. The 10 simultaneously lactating and pregnant rabbits (table 3) contained an average of 27.65 R.T.U. per pituitary, with a range of 23 to 35.50 R.T.U. per gland. Thus, there is practically no difference in the lactogen content per pituitary between the lactating and the lactating-pregnant groups. The mammary glands of the rabbits from both groups were full of milk. The average body weights (table 4) of the young from each of the two groups indicates that they were both making about the same gains.

These results show that when a rabbit is both lactating and pregnant, there is

TABLE 2. LACTOGEN CONTENT IN AP OF 20-DAY LACTATING RABBITS

No. of rabbit	Av. body weight	Pituitary weight	Units/ pituitary		Units/mg. pituitary tissue		Units/100 gm. body weight	
	gm.	mg.	R.T.U.	I.U.	R.T.U.	I.U.	R.T.U.	I.U.
1	2831	43.8	24.00	1.08	0.55	0.025	0.85	0.038
2	3645	45.8	25.25	1.14	0.55	0.025	0.69	0.031
3	3275	33.9	22.50	1.01	0.61	0.037	0.69	0.031
4	3386	37.3	27.55	1.24	0.74	0.033	0.81	0.036
5	3808	47.5	28.00	1.26	0.59	0.027	0.74	0.033
6	4558	55.3	28.50	1.28	0.52	0.023	0.63	0.028
7	3620	31.6	30.00	1.35	0.95	0.043	0.83	0.037
8	4008	33.0	26.50	1.19	0.80	0.036	0.66	0.029
9	3402	42.3	31.25	1.41	0.74	0.033	0.92	0.041
10	3641	39.8	27.50	1.24	0.69	0.031	0.75	0.034
Av.	3617	41.03	27.10	1.22	0.67	0.031	0.74	0.033

no reduction in the lactogenic hormone to the pregnancy level or cessation of milk secretion as might be expected from the inhibition theories presented. If there were factors present during pregnancy which inhibited lactogen production by the pituitary and milk secretion, they should inhibit them just as well during a period of simultaneous pregnancy and lactation. It is obvious that pregnancy does not interfere with lactation so long as the demands for nutrients by both the fetuses and mammary glands are met. If the demands made on the mammary glands for milk are reduced, as was done in these experiments by reducing the size of each litter to 2, then both lactation and pregnancy can proceed together.

Additional proof that the ovaries do not inhibit lactation is presented in table 5. It will be seen that all 4 ovariectomized rabbits failed to increase appreciably in lactogen content per pituitary. Their pituitaries averaged 10.60 R.T.U. per gland, which is not significantly different from that of the 20-day pregnancy group (table 1). Involution of the fetuses occurred in the first 3 pregnant does, and the fourth doe aborted. Although the mammary glands of these pregnant rabbits were fully developed, only a slight amount of milk was found after ovariectomy.

The extracts made from the placentas of 3 of the pregnant lactating rabbits all gave positive results when injected intracutaneously over the crop glands of pigeons. This confirms the findings of Ehrhardt (9) and Lessmann (10). It is impossible to say

TABLE 3. LACTOGEN CONTENT IN AP OF 20-DAY LACTATING-PREGNANT RABBITS

No. of rabbit	Av. body weight	Pituitary weight	Units/ pituitary		Units/mg. pituitary tissue		Units/100 gm. body weight	
	gm.	mg.	R.T.U.	I.U.	R.T.U.	I.U.	R.T.U.	I.U.
1	2891	33.0	23.50	1.059	0.70	0.032	0.81	0.036
2	4010	44.0	24.50	1.104	0.56	0.025	0.61	0.027
3	3805	53.8	29.75	1.340	0.55	0.025	0.78	0.035
4	3725	43.0	22.75	1.025	0.53	0.024	0.61	0.027
5	4294	63.4	30.50	1.372	0.48	0.022	0.71	0.032
6	3990	44.8	25.75	1.159	0.57	0.026	0.65	0.029
7	4183	31.5	26.50	1.193	0.84	0.038	0.63	0.028
8	4355	44.5	35.50	1.599	0.80	0.036	0.83	0.037
9	3877	45.7	23.00	1.036	0.50	0.023	0.59	0.027
10	3270	36.5	34.75	1.565	0.95	0.043	1.06	0.048
Av.	3840	44.02	27.65	1.245	0.648	0.029	0.728	0.032

TABLE 4. BODY WEIGHTS OF YOUNG AT 20 DAYS POSTPARTUM

20-day lactating rabbits		20-day lactating-pregnant rabbits	
No. of rabbit	Wt. of 2 young	No. of rabbit	Wt. of 2 young
	gm.		gm.
1	641	1	576
2	1118	2	722
3	855	3	868
4	810	4	519
5	965	5	921
6	769	6	850
7	760	7	889
8	930	8	1052
9	505	9	764
10	824	10	711
Av.	806.7	Av.	787.4

at the present time whether this represents an actual secretion of the lactogenic hormone by the placenta or only a storage.

DISCUSSION

It is suggested that the principal reason no substantial lactation occurs during pregnancy is that there is a low lactogen content in the pituitary at that time. Quantitative work conducted in this laboratory on the lactogenic hormone of the pituitary has demonstrated that lactation is intimately associated with a considerable augmentation of the lactogenic content of the pituitary. This increase in the lactogen content following parturition has been shown to be independent of the suckling stimulus (16). Only recently, in this laboratory, a case of spontaneous lactation was observed in a mature non-pregnant rabbit. An assay of her hypertrophied pituitary revealed that it contained 48 R.T.U. of lactogen which is about four times the quantity present in a normal mature non-lactating female rabbit.

There is no suppression either of the lactogenic hormone or lactation during a period of simultaneous lactation and pregnancy. If the inhibition theories were valid, then the lactogenic hormone in the pituitaries of such animals should be reduced and lactation should cease. It also appears unlikely that the ovaries through their secretions inhibit lactation during pregnancy. Removal of the ovaries in the last third of pregnancy does not bring about any significant change in the lactogen content of the pituitary or initiate a copious lactation. The mechanism responsible for the significant increase in the lactogenic hormone of the anterior pituitary following parturition, upon which depends the initiation of lactation, remains unsolved.

It is suggested that lactation such as normally occurs after parturition is possible only when two conditions are fulfilled (*a*) a well developed mammary gland and (*b*)

TABLE 5. LACTOGEN CONTENT OF AP OF RABBITS OVARIECTOMIZED ON 20TH DAY OF PREGNANCY

No. of rabbit	Day of killing	Body wt.	Pituitary wt.	Units/ pituitary		Units/mg. pituitary tissue		Units/100 gm. body wt.	
		gm.	mg.	R.T.U.	I.U.	R.T.U.	I.U.	R.T.U.	I.U.
1	48 hr. post-op.	3101	21.80	10.25	0.46	0.47	0.021	0.33	0.015
2	48 hr. post-op.	3580	33.00	11.15	0.50	0.34	0.015	0.31	0.014
3	5 days post-op.	3283	27.20	11.00	0.49	0.40	0.018	0.33	0.015
4	5 days post-op.	3150	24.90	10.50	0.47	0.42	0.019	0.33	0.015
Averages		3278	26.72	10.72	0.48	0.407	0.018	0.32	0.0147

a high rate of lactogen secretion by the pituitary. Of course this does not mean that lactation is impossible if either condition is only partially fulfilled. Some lactation may occur in a partially developed mammary gland (i.e., a duct system) if sufficient lactogen is present, although the reverse situation is perhaps less apt to occur. On the other hand these two conditions must be met if copious lactation is to be initiated.

SUMMARY

The following theories, based on experimental evidence, have been offered to explain the absence of lactation during pregnancy and its initiation following parturition: (*a*) the placenta produces a suppressing agent during pregnancy which holds lactation in check; (*b*) the mechanical distension of the uterus by the fetuses and placentas inhibits lactation; (*c*) the secretion of progesterone by the corpora lutea suppresses lactation during pregnancy and (*d*) during pregnancy the comparatively large amounts of estrogen present in the blood stream inhibit the lactation process. The sudden removal of the inhibition at parturition, due to the diminution of estrogen, is then supposed to provide the stimulus for lactation.

The lactogenic hormone of the anterior pituitary has been shown to be initimately associated with lactation, a factor which most of the above theories fail to take into account. Quantitative studies conducted in this laboratory on the lactogen content of the pituitary have demonstrated the important fact that this hormone remains low during pregnancy but increases 2- to 4-fold following parturition. It is suggested, therefore, that the primary reason lactation does not occur during pregnancy is because the lactogenic hormone in the pituitary is too low to support lactation. The cause of the increased elaboration of lactogenic hormone following parturition is unknown at present.

In these experiments it was demonstrated that pregnancy in rabbits had no effect on the lactogenic hormone. On the 20th day postpartum the lactogen content of the pituitaries of 10 rabbits during a period of simultaneous pregnancy and lactation was just as high (27.65 R.T.U. or 1.24 I.U.) as in 10 rabbits which were only lactating (27.10 R.T.U. or 1.22 I.U.). When rabbits were ovariectomized on the 20th day of pregnancy and killed 2 to 5 days later, their average lactogen content per pituitary (10.60 R.T.U. or 0.48 I.U.) was not significantly different from the amount present in the pituitaries of intact animals on the 20th day of gestation (9.75 R.T.U. or 0.44 I.U.). The placentas of 3 of the pregnant-lactating group were shown to contain lactogenic hormone. These results are believed to prove that pregnancy has no inhibitory effect on the lactogenic hormone of the pituitary.

It is suggested that copious lactation can be initiated only when (*a*) there is a well developed mammary gland present and (*b*) when there is a high lactogen content in the anterior pituitary.

REFERENCES

1. Hildebrandt, P.: *Beit. z. Chem. Physiol. u. Path.* 5: 463. 1904.
2. Halban, J.: *Arch. f. Gynäk.* 75: 353. 1905.
3. Frankl, O.: *Am. J. Obst. & Gynec.* 6: 399. 1923.
4. Smith, G. van S., and W. O. Smith: *Am. J. Physiol.* 103: 356. 1933.
5. Nelson, W. O.: *Endocrinology* 18: 33. 1934.
6. Litt, S.: *Am. J. Obst. & Gynec.* 26: 37. 1933.
7. a. Selye, H., J. B. Collip and D. L. Thomson: *Proc. Soc. Exper. Biol. & Med.* 30: 589. 1933.
   b. Selye, H., J. B. Collip and D. L. Thomson: *Proc. Soc. Exper. Biol. & Med.* 31: 82. 1933.
8. Newton, W. H., and N. Beck: *J. Endocrinol.* 1: 65. 1939.
9. Ehrhardt, K.: *München. med. Wchnschr.* 29: 1163. 1936.
10. Lessmann, F.: *Ztschr. f. Geburts. u. Gynäk.* 119: 271. 1939.
11. Selye, H., J. B. Collip and D. L. Thomson: *Endocrinology* 18: 237. 1934.
12. Hammond, J.: *Proc. Roy. Soc.* 89: 534. 1917.

13. Drummond-Robinson, G., and S. A. Asdell: *J. Physiol.* 61: 608. 1926.
14. Selye, H., J. B. Collip and D. L. Thomson: *Proc. Soc. Exper. Biol. & Med.* 30: 588. 1932.
15. Anselmino, K. J., and F. Hoffmann: *Zentralbl. f. Gynäk.* 60: 501. 1936.
16. Reece, R. P., and C. W. Turner: *Mo. Agr. Exper. Sta. Res. Bull.* #266. 1937.
17. Folley, S. J., and S. K. Kon: *Proc. Roy. Soc.* 124: 476. 1937.
18. Parkes, A. S., and C. W. Bellerby: *J. Physiol.* 62: 301. 1926–27.
19. de Jongh, S. E.: *Acta brev. neerl. Physiol.* 3: 52. 1933.
20. Robson, J. M.: *Quart. J. Exper. Biol.* 36: 153. 1935.
21. Wiegand, M.: *Zentralbl. f. Gynäk.* 61: 2391. 1937.
22. Folley, S. J., and S. K. Kon: *Proc. Roy. Soc.* 124: 476. 1938.
23. de Fremery, P.: *Vlaamsch Geneesk. Tijdschr.* 37, 1934.
24. Folley, S. J.: *Biochem. J.* 30: 2262. 1936.
25. Waterman, L., J. Freud and N. Vos-de-Jough: *Acta brev. neerl. Physiol.* 6: 84. 1936.
26. Steinach, E., M. Dohrn, W. Schoeller and W. Faure: *Arch. f. d. ges. Physiol.* 219: 306. 1928.
27. Laqueur, E., E. Brochardt, E. Dingemanse and S. E. de Jongh. *Deutsche. Med. Wchnschr.* 54: 465. 1928.
28. Fellner, O. O.: *Klin. Wchnschr.* 10: 1164. 1931.
29. de Jongh, S. E., and E. Dingemanse: *Arch. neerl. Physiol.* 16: 288. 1931.
30. Nelson, W. O.: *Physiol. Rev.* 16: 488. 1936.
31. Turner, C. W., and E. T. Gomez: *Mo. Agr. Exper. Sta. Res. Bull.* #206, 1934.
32. Folley, S. J., H. M. Scott Watson and A. C. Bottomley: *J. Physiol.* 98: 15. 1940.
33. Lewis, A. A., and C. W. Turner: *Proc. Am. Soc. An. Prod.* 33: 63. 1940.
34. Folley, S. J.: *Lancet* 1: 40. 1941.
35. Stricker, P., and F. Grueter: *Presse med.* 37: 1268. 1929.
36. Holst, S., and C. W. Turner: *Proc. Soc. Exper. Biol. & Med.* 42: 479. 1939.
37. Hoffmann, F.: *Zentralbl. f. Gynäk.* 61: 35. 1937.
38. Meites, J., and C. W. Turner (unpublished).
39. Meites, J., A. J. Bergman and C. W. Turner: *Proc. Soc. Exper. Biol. & Med.* 46: 670. 1941.
40. Bates, R. W., and O. Riddle: *J. Pharmacol & Exper. Therap.* 55: 365. 1935.
41. Meites, J., A. J. Bergman and C. W. Turner: *Endocrinology* 28: 707. 1941.

VARIATIONS IN THE YIELD OF GONADOTROPIC MATERIAL FROM GREEN PLANTS IN RELATION TO THE SEASON OF GROWTH AND THE pH OF THE FRESH JUICE[1]

MAURICE H. FRIEDMAN[2] AND JOHN W. MITCHELL

From the Bureau of Dairy Industry and the Bureau of Plant Industry, United States Department of Agriculture

BELTSVILLE, MARYLAND

IN SOME EARLIER EXPERIMENTS (1) attempts were made to prepare gonadotropic extracts from dehydrated leaves of alfalfa and immature oat plants. The extracts prepared from some samples of these plants were active, but those from many other samples were inactive. At best the yields of gonadotropic material, as measured by the rabbit ovulation test, were unsatisfactory. They were not greater than 1 ovulating dose for every 30 gm. of dried leaf[3] and were more commonly 1 dose for each 150 gm. More recently, a yield of 1 ovulating dose for every 4 gm. of dried leaf (2) was obtained from young oats.[4] The potency of this preparation, however, was not much better than the potency of the earlier extracts; i.e., about 200 mg. per ovulating dose.

By using the juice of young oat leaves, which was available from a commercial laboratory in Missouri,[5] it was possible to prepare much more satisfactory gonadotropic extracts. Although the juice was not subjected to the extraction process until after some weeks or months of storage in the frozen condition, some samples gave yields of 20 to 60 ovulating doses per liter and the final products produced ovulation in doses of 50 to 75 mg. (2). Many of these samples of oat juice, however, were completely inactive. It thus became obvious that further progress could not be expected until it was possible to eliminate the uncertainties caused by the unavoidable and variable period of storage in the frozen condition (between the time the fresh juice was expressed from the plants grown in a distant state and the time the extraction process was initiated in our laboratory).

The present experiments, with a number of plants grown in the field or greenhouse at Beltsville were undertaken to eliminate the uncertainties mentioned above and to discover, if possible, some relation between the gonadotropic activity of plant juice and some other physiological or morphological characteristic of the plant.

Received for publication April 9, 1941.

[1] This research was supported by an appropriation from Bankhead-Jones funds (Bankhead-Jones Act of June 29, 1935).

[2] Resigned, August 1940.

[3] Throughout this report the word, leaf, will be used to designate the entire above-ground portion of the immature plant. At the stages at which the plants were used the above-ground portion was almost entirely leaf, but in some cases a very small amount of stem may have been included in the sample.

[4] This sample was from oats grown on the experimental farm at the Beltsville Research Center, Beltsville, Md., and was grown especially for us by Mr. Thomas W. Moseley, Superintendent, Bureau of Dairy Industry Experiment Station, Beltsville, Md.

[5] The samples of frozen plant juice were supplied by The American Butter Company, Kansas City, Mo.

METHODS

A. Cultivation of plants and the selection of samples. The field crops were grown at the U. S. Horticultural Station at Beltsville, Md., in 1939 and 1940. The soil, which was somewhat sandy, was not fertilized in the spring of 1939 but was heavily fertilized in the 1940 season with commercial fertilizer. The field was divided into several plots which were planted consecutively at intervals of about a week or 10 days, so that samples of the same age could be obtained repeatedly at will.

Even in a single plot, plants of the same planting showed considerable individual variation in the rate of growth and development. Hence in collecting the samples an

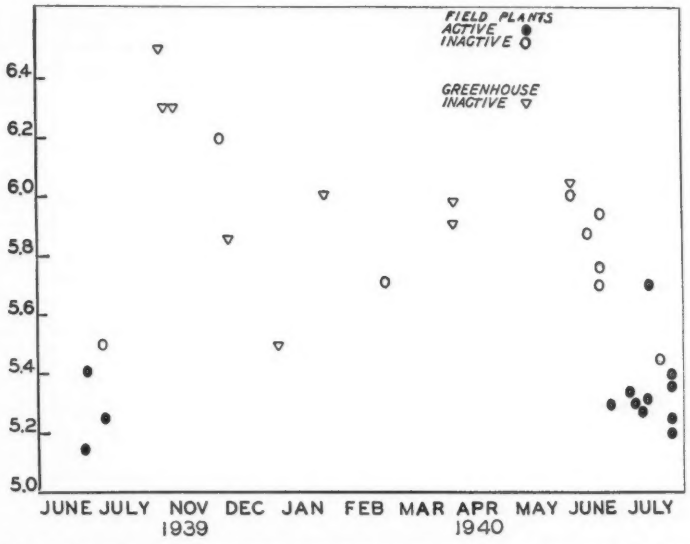


Fig. 1. The initial pH of the fresh juice from leaves of sudan grass, corn and oat plants. The samples designated as inactive were those which yielded less than 10 (or less than 5, cf. text) ovulating units per liter of fresh juice. In addition to the samples shown in this figure there were 9 others on which we neglected to make pH readings initially. These 9 were collected between November 1939 and June 1, 1940, and were all inactive.

effort was made to select plants representing the mean stage of development for the particular plot. To attain that end plants which approximated the average height were collected from all portions of the plot. The selected plants were pulled up, the root portion cut off, and the above-ground portions placed in a wet sack for immediate transfer to the laboratory. Here they were ground in a power mill before being placed in a hand press where the juice was expressed through a cotton sack. In the grinding process the temperature of the sample reached 40° to 43° C. Usually not more than 2 hours elapsed between the collection of the samples in the field and the beginning of the extraction process on the expressed juice.

The greenhouse plants were grown on compost soil. The temperature was not constant, but varied from 70° to 85° F. during September and from 60° to 80° during the winter months. Except in a few exploratory experiments to be mentioned later the plants were grown in the natural light that filtered through the greenhouse glass. The day length varied from 13½ hours in September to 9½ hours in January.

B. Extraction process. With few exceptions the pH of the freshly expressed juice was measured as soon as it was obtained (before any chemical treatment). These readings are referred to as the initial pH and are the ones shown in figure 1. The juice was then passed through a Sharples centrifuge at about 50,000 r.p.m. for clarification, and the resulting fluid adjusted to pH 5.0 before precipitation by the benzoic acid method previously described (1). In the present experiments the washings with saturated aqueous benzoic and were omitted and the number of washings with cold acetone was increased. The exact number of these washings varied from 4 to 10, depending upon the nature of the precipitate and the effort required to remove all traces of acetone-soluble pigment and the excess of benzoic acid. The precipitate was then dried at room temperature under reduced pressure and dehydrated for at least 24 hours over phorphorus pentoxide.

The bulk of the dried precipitate varied with the initial pH of the plant juice. The precipitate obtained from samples with a low initial pH weighed between 2 and 4 gm. per liter of juice and could be assayed directly without further chemical fractionation. The precipitate obtained from samples with a high initial pH was so bulky that it was necessary to resort to the 30% acetone fractionation (1) before assay in order to reduce the amount of solid matter in the final product. In such instances the combined 30% acetone extracts were adjusted to pH 5.0, instead of pH 6.0 as in the earlier experiments (1), and poured into 8 volumes of cold acetone. The resulting precipitate was dried and dehydrated in the usual manner. In all instances the final product was stored in the cold in small desiccators over phosphorus pentoxide until assayed. The interval of storage until the first assay was usually no more than 3 days and never exceeded 6 days. The most significant change from our earlier work was the substitution of the glass electrode for indicators in all pH readings.

C. Assay. At the time of assay the final powders were subjected to an extraction in dilute sodium hydroxide at a pH of 7.4 to 7.6 for about 2 hours. Any insoluble matter was removed by centrifuging and the solution then made up to a volume of about 50 cc. for assay in on the postpartum rabbit (3).

For the present experiments it was decided to label as inactive any product which failed to produce ovulation in doses equivalent to 100 cc. of original plant juice (i.e. one-tenth of the solution made from the entire yield of 1 liter of plant juice). In many instances the final product was of such low toxicity that it was possible to inject much larger doses without unfavorable effects in the assay animals, so that for one-half of the inactive samples obtained in the winter and spring, the negative assays indicated a potency of less than 5 ovulating doses per liter.

The phenomenon of the zone effect made it necessary to take special precautions in our biological tests. This phenomenon was first encountered in the assay of crude extracts of pregnancy urine (3). Such extracts, in contrast to more highly purified ones, were obviously toxic in doses 100 times the minimal effective dose, and at these high levels gave negative instead of positive ovulation tests. The same phenomenon was encountered in these experiments with plant extracts. For example, one corn extract was lethal at doses equivalent to 120 cc. of fresh juice and gave negative responses at equivalents of 50 and 40 cc. Below these levels a whole series of positive responses was obtained down to an equivalent of 3 cc. of fresh juice. The extent of the dose range over which it was possible to obtain positive responses varied with the toxicity of the product. With the best product it was possible to secure an unbroken series of positive responses when using 1 to 15 times the minimal effective dose. With some extracts, however, negative responses were obtained with as little as 5 times the minimal effective dose. To rule out errors arising from the zone effect it was necessary to assay not only with doses of the order of one-tenth of the final

product, but to check any negative results with a series of injections from this level down to 0.01 to 0.02 of the yield from 1 liter, even in the absence of any signs of toxicity. When the extracts were obviously toxic in the higher doses, even greater care was taken to avoid the zone effects, and the dose range was carried down still farther, in some instances to the equivalent of 0.003 of the final product from one liter of fresh juice.

RESULTS

During the summer of 1939 tests were performed on the leaves of a number of different plants, including feterita, corn, kidney beans, Amber sorghum, Sudan grass, German millet, rape, soy bean and alfalfa.[6] Of the 14 samples representing these 9

TABLE 1. THE YIELD OF GONADOTROPIC ACTIVITY FROM THE JUICE OF THE CORN PLANT IN RELATION TO THE AGE AND HEIGHT OF THE PLANT AND INITIAL PH OF THE JUICE

Date[1]	Sample no.	Age[2]	Height[3]	Initial pH	Weight of ppt.[4]	Yield of activity ovulating doses/l.[5]
		days	*in.*		*gm.*	
1940						
June 24	40	28	10–12	5.30	2.9	290
July 1	41	26	10–12	5.35	2.9	15
July 8	42	42	10–12	5.30	4.0	13
July 8	43	42	14–18	5.28	3.3	12
July 10	44	29	10–12	5.32	2.9	15
July 16	46	35	10–14	5.45	3.5	0
July 23	49	13	3–4	5.35	2.9	12
July 23	50	21	9–10	5.40	4.2	12
July 23	51	40	18	5.25	4.0	35
July 23	52	61	36	5.20	1.9	25

[1] Date on which samples were harvested.
[2] From time of planting to harvesting.
[3] Height of above-ground portion of the plant.
[4] Weight of washed and dried benzoic acid precipitate per liter of juice.
[5] Yield of gonadotropic activity.

plants only 4 yielded potent products. Two were from corn, and one each from feterita and Sudan grass. Since potent extracts had previously been obtained from alfalfa and oat leaves (1) it was apparent that the gonadotropic substance was not confined to any one plant genus. Moreover, the presence of biologic activity in some samples and its absence in other samples of the same species emphasized the fact that negative results with a few isolated samples of one kind of plant had little or no meaning. There seemed to be no reason for dissipating our energies by analyzing a great variety of plants. Instead, we focused our attention on oats, corn and Sudan grass, with most of the emphasis on the first two.

A survey of the results with these three species reveals the presence of gonadotropic activity in the plant samples harvested in late June and July of 1939, and again in the samples harvested in late June and July of 1940, but not in the samples of the intervening months. During this period, from November of 1939 until the third week in June of 1940, 25 separate samples were tested, some in duplicate or triplicate. The biological assays on these samples were consistently negative regardless of whether the plants had been grown in the field or in the greenhouse[7] (fig. 1).

The gonadotropic potency was not the only difference between the summer plants

[6] With the exception of the soy bean plants, which were grown in the greenhouse, all of these plants were grown in the field.
[7] Measurements of initial pH were made on only 16 of the 25 samples collected during this period.

and those obtained in the winter and spring. In general, the initial pH of the plant juice was higher in the samples obtained in the winter and early spring (fig. 1). Related to this variation in pH was the yield of solids per liter in the washed and dried benzoic acid precipitate. From samples with an initial pH of 5.4 or less the yield was between 2 and 4 gm. per liter of juice. Above pH 5.5 the yield or solids was rarely below 7 gm. and usually between 10 and 15 gm. per liter. Samples with an initial pH of 5.8 to 6.0 gave even higher yields, frequently exceeding 25 gm. per liter.

The age distribution of the plants from November of 1939 to June 1940 was not exactly the same as that of the July samples. Most of the inactive samples obtained during the winter and spring (until June 24, 1940) were from 25 to 35 days old and approximately 6 to 10 inches tall, but some of the samples were older and taller (up to 60 days of age and 18 inches tall). However, the age of the plant seems to be relatively unimportant, for after the reappearance of gonadotropic activity in the last week of June 1940, potent extracts were obtained consistently from the field samples regardless of the age or height of the plant. This may be seen in table 1 which shows the results with field corn only, and in which the only variables are the age and development of the plant, and time.

DISCUSSION

In a few exploratory experiments an attempt was made to alter the characteristics of the greenhouse plants by lengthening the daily light period, supplementing the ordinary lighting with extra light from a battery of electric light bulbs or a battery of sun lamps (providing some ultra-violet light), or, by maintaining a relatively low night temperature. These measures had no noticeable effect on the initial pH of the fresh juice, the weight of the benzoic acid precipitate, or the gonadotropic activity. Of course, these few experiments do not necessarily indicate that the greenhouse environment has some peculiar defect other than light or temperature. Other plants of the same kind, grown under field conditions during the same period of time were also inactive (fig. 1) and closely resembled the greenhouse samples in initial pH and yield of solids per liter of juice. Regardless of whether the plants were grown in the field or in the greenhouse we were not able to prepare satisfactory gonadotropic extracts from plants obtained in the winter or early spring. This could be explained by any one of three possibilities; *a*), that there is a true seasonal variation in the concentration of the gonadotropic material in the fresh plant juice, *b*), that our extraction methods failed consistently in dealing with every sample obtained from November of 1939 until late June of 1940, or, *c*), that there was a seasonal variation in the sensitivity of our assay animals.

The last possibility can be dismissed briefly. It had already been shown (2) that the rabbit displays no detectable seasonal variation in its response to other gonadotropic materials, such as pregnancy urine extracts and extracts from pregnant mare serum. During the course of the present experiments the animals of our colony were tested with plant extracts which had been prepared in the preceding season and which had been stored as powders. With such stored powders it was possible to obtain positive responses in every month from January to May inclusive, although it must be admitted that our assays indicated some degree of deterioration in all but one of the powders.

Although a true seasonal variation in the concentration of gonadotropic material appears to be the more likely explanation of our results, the other possibility, that our methods were at fault, cannot be dismissed without some consideration. The much higher pH values and yields of solids associated with winter and spring plants are

evidence that their composition was markedly different from that of the summer plants. It is conceivable that this change in chemical composition could adversely influence the yield of gonadotropic activity even if the concentration of the latter remained relatively constant. Yet, if we are to attribute our monotonously negative results with the fall, winter, and spring plants to the shortcomings of our methods, the failure must be charged not only to the use of the 30% acetone fractionation, but to other procedures as well. Although we routinely used this fractionation on the samples that yielded bulky precipitates, there were several occasions when the fresh juice was divided into two or three parts to be extracted and assayed separately. One part was always subjected to the 30% acetone fractionation, but the remaining portions were subjected to some modified procedures. It was found, for example, that the bulk of the washed and dried benzoic acid precipitate could be greatly decreased if the fresh juice was allowed to stand overnight in the cold room before clarification. It was also found that a similar reduction in solids could be obtained if the juice was adjusted to pH 5.0 before clarification in the centrifuge. In either event the first precipitate could be assayed directly without the additional steps of the 30% acetone fractionation.

Such modifications, however, did not alter our biological results; they were regularly negative during the winter months regardless of the extraction technic. It is possible that each of these modifications would result in some loss of activity. We have no data whatsoever on the loss, if any, caused by overnight storage in the cold, or by acidification of the juice before clarification. We do have quantitative results on the 30% acetone fractionation of two plant products of relatively great bulk and low potency. With these two products the 30% acetone fractionation made possible the removal of over 65% of the original solid matter, with a loss of less than 30% of the original activity (if any). These yields may have been particularly fortunate rather than the average to be expected for plant products, but they are in accord with our experience with other gonadotropic materials (i.e., PU or anterior lobe powders). In any event the use of 30% acetone fractionation did not prevent us from detecting gonadotropic activity in the samples of dried leaves used in the earlier experiments (1, 2), in several samples of oat juice which had been stored in the frozen condition and sent by express from Kansas City to Beltsville, and in the only sample (oats) of July 1940 which required this treatment (see figure 1, the active sample with a pH of 5.9).

Irrespective of the true cause for the negative results throughout the fall, winter and early spring, our data very strongly suggest some relation between the yield of gonadotropic activity and the initial pH of the plant juice. It is striking that gonadotropic activity did not reappear in our extracts until there was a very sharp drop in pH late in June 1940. With one exception, a line through pH 5.4 (fig. 1) separates the active from the inactive samples. This relation between pH and the yield of active material may prove to be a valuable guide in the search for more suitable plant samples. But this would not necessarily prove any fundamental relation between pH and those plant processes responsible for the production of the gonadotropic substance. It should be noted that below pH 5.4 tremendous variations in the yield of active material are encountered without significant variations in pH, so there are probably other factors of greater importance. Although there is no clue as to the identity of these factors, the age of the plant and its stage of development do not seem to be among them.

Further progress in this line of work would be greatly facilitated if we could discover some criterion to lead us to more samples like number 40 (table 1). Because of the crudity of most of our plant products we have made no allowance for the body

weight of the rabbits used in these assays. If the potency of the plant extracts were expressed in rabbit units (amount per kilogram of postpartum rabbit required to produce ovulation), the figures in table 1 would have to be multiplied by 4 since the average weight of the assay animals was approximately 4 kg. (3). On this basis, the exceptional plant juice which furnished sample 40 yielded between 1,000 and 1,200 rabbit units per liter. This is about the gonadotropic activity of pooled urine from pregnant women during the latter part of pregnancy.

SUMMARY

With the methods employed in these experiments there was a seasonal variation in the yields of gonadotropic activity from the juice of the above-ground portion of immature Sudan grass, oat and corn plants. The inactive juices obtained from plants in the autumn, winter and spring, were characterized by a high initial pH and a high yield of solids in the washed and dried benzoic acid precipitate. The active juices from plants obtained in the summer months were characterized by a low initial pH and a low yield of solids in the precipitate. In general, the plant juices having an initial pH above 5.4 were inactive while those with an initial pH below 5.4 were active. Below this critical pH, variations in the acidity of the juice were not correlated with corresponding variations in the yield of gonadotropic activity. Most of the active samples were of relatively low potency, averaging about one twentieth of the titer of the late pregnancy urine. One sample, however, was comparable to late pregnancy urine (human) in gonadotropic potency. The yield from this exceptional sample was 1,000 rabbit units per liter of fresh juice.

REFERENCES

1. Friedman, M. H., and G. S. Friedman: *Am. J. Physiol.* 125: 486. 1939.
2. Friedman, M. H., and G. S. Friedman: unpublished data, 1939.
3. Friedman, M. H.: *Endocrinology* 24: 617. 1939.

THE SITE OF ELABORATION OF THE PITUITARY GONADOTROPIC HORMONE AND OF PROLACTIN[1]

MAURICE H. FRIEDMAN[2] AND S. R. HALL

From the Division of Nutrition and Physiology, Bureau of Dairy Industry, United States Department of Agriculture

BELTSVILLE, MARYLAND

THE PRESENT FOCUS of attention on the attempts to fractionate pituitary extracts into several relatively pure substances, each possessing only one characteristic hormonal action, has emphasized the chemical individuality of several of the endocrine activities of the anterior lobe. While there may be some question as to details of biological characterization, and some dispute as to the precise number of separate anterior lobe hormones, there is complete agreement that the phenomena of reproduction, growth and lactation are dominated by different humoral agents.

The physiological processes of growth, reproduction and lactation do not necessarily proceed concurrently and at the same rate. Indeed, in the early part of life the growth process is proceeding at its maximum while the organs of reproduction and lactation are showing very little activity. On the other hand, the processes of growth, reproduction and lactation can proceed simultaneously. We must, therefore, credit the pituitary with the ability to liberate at least some of its hormones at independently variable rates. It would be easy to account for such an ability if each separate hormone were produced by a cell type devoted exclusively to that function. However, the postulated number of separate anterior lobe hormones far exceeds the number of different cell types now recognizable. Unless the actual number of hormones is much smaller, we are obliged to question the adequacy of present histological technic, or to assume that each cell in the anterior lobe secretes more than one hormone. In the latter event, the secretion of each pituitary hormone at an independently variable rate would be impossible unless the individual cell could release one of its hormones at any desired rate while retaining the others.

The experiments we are now reporting were designed to find out if prolactin and the gonadotropic hormone(s) were produced by the same cell or by different cells, and whether a maximal secretory rate of one hormone could be attained without accelerating the secretion of the other.

METHODS AND MATERIALS

Beef glands. These were obtained from a local slaughter house[3] in groups of 24 to 50 glands for each experiment. As soon as the glands were dissected out of the head they were placed in a vessel encased in carbon dioxide snow for transport to the laboratory. Usually the interval between the slitting of the animal's throat and the time the gland was frozen solid in the cold chamber did not exceed 30 minutes. As each

Received for publication May 13, 1941.

[1] This research was supported by an appropriation from Bankhead-Jones funds (Bankhead-Jones Act of June 29, 1935).

[2] Resigned August 8, 1940.

[3] For help in obtaining these glands we wish to thank Mr. G. B. Aspinwall of the Corkran, Hill & Company, Inc., Baltimore, Md.

gland was removed from the cold chamber in the laboratory it was drilled through, antero-posteriorly, with a small cork borer, 3.3 to 6.0 mm. in diameter. Thus, from each gland there was available a long, cylindrical plug of central tissue, a medulla; and the remaining peripheral portion, or cortex. In 2 experiments the latter was subjected to a second drilling with a larger cork borer to remove an intermediate zone concentric to the medullary plug. After these preparations, the medullary, intermediate and cortical portions, respectively, were pooled and placed in separate containers under an excess of cold acetone for desiccation. They were then dried under reduced pressure at room temperature. The dry glandular material was powdered manually in a mortar, after which the coarser fibrous matter was discarded by screening through an 80-mesh sieve. Until the time of the first assay, some 2 to 5 days later, the powders were stored over phosphorus pentoxide in the cold.

Rabbit glands. The 10 to 20 donors of the rabbit pituitaries used for each experiment were anesthetized with nembutal and exsanguinated. While the blood was still flowing, the pituitary glands were removed in one piece and placed in a thick-walled centrifuge tube under an excess of acetone. During the period of 2 to 5 days which elapsed before the assay, the glands were stored in these tubes in the refrigerator.

For donors, we selected females which had delivered a litter of healthy young, 3 to 4 weeks previously, or which had ovulated 3 to 5 weeks previously either in response to sterile coitus, or to an injection of a gonadotropic extract. Since the postpartum animals had not been allowed to nurse their young, it is possible to characterize all of the donors in the following manner: *a*) they were estrous rabbits in which the mammary glands had recently undergone considerable development; *b*) they were not lactating at the time of the experiment; *c*) they had not been secreting significant quantities of milk for 3 to 4 weeks preceding the time the glands were obtained. Nevertheless, inasmuch as we were not certain that the post-pseudopregnant rabbits were comparable to the postpartum animals with respect to the prolactin content of the pituitary, we made an effort to include in each group of donors the 2 types of animals in approximately the same proportions.

Extraction procedures. Unfortunately, we were not able to use the same extract for the assay of both the gonadotropic and prolactin activity. We had found that the maximum yields of gonadotropic activity were obtained by extraction at pH 8.3, whereas the maximum yield of prolactin activity was obtained at a pH of about 9.5.

For the prolactin assays the powdered material from the beef glands was extracted for about 1 hour in water adjusted to a pH of 9.5 by the addition of N/20 sodium hydroxide, using thymol-phthalein as an indicator. The suspension was then centrifuged and the tissue residue subjected to another extraction at this same pH for 1 hour. In the second extraction, however, sodium chloride was added in a quantity sufficient to bring the concentration of this salt to about 2%. At the end of this second extraction the tissue residue was discarded by centrifuging. The clear supernatant fluid was combined with that of the first extract and neutralized with dilute hydrochloric acid. As a final step the extract was diluted with distilled water to bring the concentration of sodium chloride down to 1%. Each cubic centimeter of such an extract represented the biologic activity of 4 to 6 mg. of dried beef pituitary. Repeated assays on the solid matter left behind after this process indicated a prolactin content of not more than 2% (if any) of that found in the extract.

The rabbit glands were dried under reduced pressure and ground to a powder with a stout glass rod without removing them from the thick-walled centrifuge tube. They were then treated exactly like the powdered material from the beef glands. In the case of the rabbit glands, however, the final volume was adjusted so that each cubic centimeter of the extract represented about one-half of 1 rabbit pituitary.

For the gonadotropic assays, the powdered material from the beef glands was sub-

jected to 2 one-hour extractions at a pH of about 8.3, using phenolphthalein as an indicator. Sodium chloride was not added during this process, but only during the final adjustment of volume when enough 10% sodium chloride was added to bring the salt concentration to about 1%. In these extracts, 1 cc. of fluid represented 1 mg., or less, of dried beef pituitary.

Prolactin assays. For the determination of prolactin activity we employed the systemic crop gland technic of Riddle and Bates (1), using exclusively White Carneau pigeons of uniform age (about 6 weeks after hatching) and weight (about 450 gm.). In an extensive series of preliminary experiments we established a dose-response curve for such crude pituitary extracts as we were using. This curve was almost identical with the curve published by Riddle and Bates for prolactin preparations.

For all of our assays we injected 0.5 cc. daily for 4 days. The total volume injected intramuscularly over the 4-day period was, therefore, 2.0 cc. and represented 8 to 12 mg. of dried beef pituitary, or, about 1 rabbit pituitary. The prolactin activity was then determined by converting the average crop gland response into Riddle-Bates units by reference to the dose-response curve. With the exception of 1 experiment on beef pituitary, when only 6 birds were used, the prolactin activity of each extract was determined by the average crop gland response of 13 to 15 pigeons.

Gonadotropic assays. The assays on beef pituitary powders were conducted by the rabbit ovulation method (2) using not less than 4 post partum rabbits for each critical dose level.

RESULTS

Distribution of prolactin and gonadotropic hormone in bovine glands. The results of these experiments are summarized in table 1. In each of the 5 experiments the prolactin content of the medullary portion was lower than that of the cortical portion.

TABLE 1. THE DISTRIBUTION OF GONADOTROPIC HORMONE AND PROLACTIN IN THE BOVINE PITUITARY[1]

Experiment No.	Av. pituitary weight	Part[2]	Diameter	Relative weight	Assay	
					Gonad	Prolactin
	mg.		mm.	%		
1	220	M.	3.3	9.5	8.0	0.6[3]±0.07
(24 steers)		I.Z.	8.6	52.0	3.3	1.2[3]±0.16
		C.	—	38.5	1.2	1.7[3]±0.35
2	348	M.	4.5	14.3	2.0	1.1±0.11
(50 steers)		C.	—	85.7	1.0	1.6±0.16
3	220	M.	6.0	25.5	—	1.2±0.20
(25 steers)		C.	—	74.5	—	1.5±0.12
4	350	M.	3.3	6.5	1.3	1.8±0.25
(25 cows)		C.	—	93.5	1.3	2.6±0.50
5	295	M.	3.3	6.5	2.5	1.0±0.22
(24 steers)		I.Z.	8.6	32.5	—	1.4±0.25
		C.	—	62.0	0.8	1.3±0.22

[1] The results of the assays are expressed in rabbit units or Riddle-Bates pigeon units per mm. of dry tissue. The standard error of estimate shown for the prolactin assays was obtained by projecting the standard error of the mean crop gland weight onto the dose-response curve.

[2] M. = medulla, I.Z. = intermediate zone, C. = cortex.

[3] These prolactin values were obtained in assays conducted on the powders almost a year after they had been placed in storage (over phosphorus pentoxide, in the cold). The assays which were performed a few days after these powders had been obtained involved the use of subcutaneous injections. Since there was no significant difference between the results of the 2 sets of assays, and since all other prolactin assays were performed with intramuscular injections, we have elected to present the second (intramuscular) set of assays despite the long period of storage.

The difference was not great, however, varying from 1.5 to 5 times the standard error of the difference between the estimates. In the 2 experiments in which the intermetiate zone was assayed separately (*experiments* 1 and 5) the difference between it and the cortex cannot be regarded as significant.

Conversely, the gonadotropic activity of beef steer glands was much higher in the medullary portion than in the cortical portion. In these assays the differences were so much greater than the standard error of estimate (2) that they are decisive in each instance. The single experiment on pituitaries from cows (*experiment* 4), however, gave results which were not in accord with the others.

The prolactin content of the rabbit pituitary after a stimulus which induces a maximal release of gonadotropic hormone. A comparison of the prolactin content of glands from estrous animals with the prolactin content of glands taken from animals 24 hours after the stimulus of coitus is shown in table 2. It is apparent that the yield of prolactin activity from estrous glands varies from time to time, with a tendency for the higher values in the spring time. Regardless of the cause of these variations, it is obviously impossible to compare the prolactin content of one group of glands with that of another group unless the glands are obtained at about the same time and the 2 assays conducted within a very short time interval. For this reason the experiments of 1939 should not be considered very seriously. The interval between the experiment on the post-coital glands (4–1–39) and the experiment on the estrous glands (6–8–39) was probably too great. These data are included merely to emphasize the variation in yield with time. Nevertheless, the experiments conducted in May and June of 1940 yield essentially the same kind of data. The glands taken from animals 24 hours post-coitum have a prolactin content quite as high as those taken from unstimulated, estrous animals.

Control experiments on the influence of inert matter In our preliminary work on the dose-response curve for the prolactin activity of crude pituitary extracts, it was apparent that our methods would permit the detection of relatively small differences of prolactin activity. In this work, however, the variation in dosage was accomplished by the dilution of a concentrated extract; i.e., under conditions in which the ratio of active hormone to inert pituitary protein was constant. In our assays of rabbit glands this was not true. We may safely assume that total solid matter extracted from the glands was relatively constant, so that any increase or decrease in the prolactin content could cause a significant change in the ratio of hormone to inert protein con-

TABLE 2. THE EFFECT OF COITUS ON THE PROLACTIN CONTENT OF THE FEMALE RABBIT PITUITARY

Date	Donors		Prolactin
	Type	Number	
			u/pituitary
4- 1-39	post-coital	12	11.6
6- 8-39	estrous	10	10.0
6-16-39	estrous	9	8.6
2- 3-40	estrous	11	4.5
2- 4-40	estrous	13	5.5
2-26-40	estrous	15	4.5
3-25-40	estrous	14	3.4
4-22-40	estrous	15	8.9
5- 1-40	estrous	15	5.8
5-10-40	post-coital	18	5.8
5-21-40	estrous	17	8.2
6-18-40	post-coital	19	9.1
7- 2-40	estrous	18	5.6

TABLE 3. THE EFFECT OF BEEF MUSCLE EXTRACT UPON THE CROP GLAND RESPONSE TO PROLACTIN

Date 1940	Injection, total dose		Av. crop gland	Prolactin activity	
	Prolactin	Muscle		Total	Per mg.
	mg.	mg.	mg.		
4-29	1.0	—	2669	13.5±2.1	13.5
	1.0	8.0	3040	19.7±3.2	19.7
5-23	0.6	8.0	2465	10.9±1.6	18.0
	0.4	8.0	2237	8.6±1.5	21.0
	0.2	8.0	1392	3.6±0.3	18.0
6-14	1.0	—	2721	14.2±2.2	14.2
	—	8.0	1125	0.0	—

The standard error shown for each estimation of prolactin activity was calculated in the fashion noted in table 1.

tained in extract. There have been no reports to indicate that inert protein modified the crop gland response to prolactin. Yet, we felt obliged to consider this possibility and to determine whether or not our methods were capable of detecting differences in prolactin concentration when the active substance was assayed in the presence of a large and constant amount of inert matter.

To represent the inert proteins of the rabbit pituitary we made an extract of dried beef muscle, prepared by the technic used for the prolactin extractions. Because the dry weight of the rabbit pituitary is 7 to 8 mg., the beef extract was mixed with prolactin[4] solutions of varying strength in such manner that every 2 cc. of the assay solution contained the extract from 8 mg. of dried beef muscle. The results of these experiments are shown in table 3.

It is apparent that the biologic effectiveness of the prolactin was markedly enhanced by the addition of the inert beef muscle extract. Of more direct interest is the finding that a 30 to 40% decrease in prolactin concentration is detectable by our methods even when the active substance is assayed in the presence of a large and constant amount of inert matter.

It may be noted that during the period covered by these experiments the sensitivity of the assay animals did not show any significant variation.

DISCUSSION

These experiments do not represent the first attempt to establish the physiologic individuality of the several chemical entities responsible for the endocrine effects of the pituitary. The earlier experiments of Smith and Smith furnished direct evidence that the biologic effects of the medullary portion of the beef pituitary are different from those of the cortical portions (3). When tested on hypophysectomized tadpoles, the eosinophilic, cortical portion stimulated body growth without affecting the rate of metamorphosis; whereas the basophilic, medullary portion markedly stimulated the thyroid gland and accelerated metamorphosis without affecting somatic growth. Subsequently, Smith and Dortzbach (4) demonstrated a temporal separation of the growth and gonadotropic hormones in the fetal pig pituitary. In fetuses of 9 to 11 cm. in length, the growth hormone was found in the absence of detectable amounts of gonadotropic hormone. The latter first appeared in fetuses of 18 to 20 cm. in length. More recently, Schooley and Riddle (5), in addition to some interesting histological studies, presented some prolactin and gonadotropic assays on pigeon pituitaries in

[4] Prolactin was supplied by the Eli Lilly Company, Indianapolis, through the courtesy of Dr. E. D. Campbell.

various stages of somatic development and sexual activity, and on the medullary and cortical portions of bovine pituitary. Unfortunately, from the few assays presented, it is difficult to translate these data into satisfactory quantitative terms which can be subjected to an adequate statistical examination. Apart from the indirect evidence secured by a correlation of functional states with histological alterations in the pituitary cells, no other pertinent data are available.

In our own assays on beef pituitary the higher prolactin content of the cortex was not statistically significant in each of the 5 experiments. Yet, because the difference between the cortex and the medulla was consistently in one direction, the data of the 5 experiments together are statistically significant. If the prolactin content of the two portions were identical, the chances of getting a series of differences like those actually observed are less than 3 in 100. The gonadotropic assays on the same material more were decisive. An allowance of 15% for the standard error of estimate is ample (2), so that in *experiments* 1, 2 and 5, the observed difference between the two portions of the gland was from 3 to 7 times the standard error of the difference.

The discordant results of *experiment* 4 are not altogether surprising. They may be attributable to our use of cow glands, instead of steer gland in this one experiment, or, they may merely serve to emphasize the technical difficulties confronting us. Our purpose and intent was to divide the beef pituitary into cortical and medullary areas on the basis of the distribution of eosinophils and basophils. In actual practice the attainment of a sharp division on this basis is impossible. Even if we had been able to guide our dissection by differential staining, the division would have been an imperfect one. The basophils of the medulla are always invaded by variable number of eosinophils, and some basophils are scattered throughout the eosinophilic cortex. Indeed, a small densely granular basophil, distinguished by the use of hemotoxylin, is distributed evenly throughout the anterior lobe (6). When the dissection is performed by a blind operation, as in our experiments, the success of the operation is jeopardized by additonal variables. Probably the most important of these variables are the average cross-sectional area of the pituitary glands, the size, shape, and position of the basophilic area, and the deviations of the drilling from the projected line. For example, the weight of the pooled central plugs removed by the cork-borer 3.3 mm. in diameter varied from 6.5% of the gland in *experiment* 5, to 9.5% of the gland in *experiment* 1. When we look at the variation in average pituitary weight, the explanation is obvious. When the diameter of the medullary plug is constant, the relative size of the medullary area removed will vary inversely with the cross-sectional area of the gland. On the basis of our present experience, we might suggest that the sharpest contrast between the medullary and cortical areas of the beef gland will probably be obtained when the medullary area is large enough to constitute 10 to 12% of the gland by weight, and when the transitional, or intermediate zone has been excluded from the cortex.

Despite the limitations of our methods there can be little doubt that prolactin and the gonadotropic hormone(s) are concentrated in different areas of the bovine pituitary. Conceivably, such differential concentration could arise from reciprocal variations of the concentrations of the two hormones in one type of cell. In this event, however, we should hardly expect to find the gonadotropic hormone(s) consistently concentrated in the medullary area, and prolactin consistently concentrated in the cortical area. The only plausible explanation for this consistent spatial distribution is that each of the two hormones is elaborated and stored by a distinct type of cell, and that the two cell types are not distributed uniformly throughout the anterior lobe of the beef pituitary.

From the experiments on the rabbit pituitary, it is clear that a stimulus which

discharges the gonadotropic hormone(s) almost completely has no detectable effect on the prolactin content. Unless the rate of restitution of prolactin is enormously greater than that of the gonadotropic hormone, we must conclude that there is little or no acceleration of prolactin secretion during the 24 hours following coitus. In other words, we must then conclude that in the rabbit, the gonadotropic hormone can be released at a maximal rate without affecting the secretory rate of prolactin.

Currently, there is a general disposition to associate the growth hormone with the eosinophils and the gonadotropic hormone(s) with the basophils. A mass of indirect evidence in support of such association has been ably summarized by Severinghaus (7). In view of our own results on the gonadotropic hormone content of the

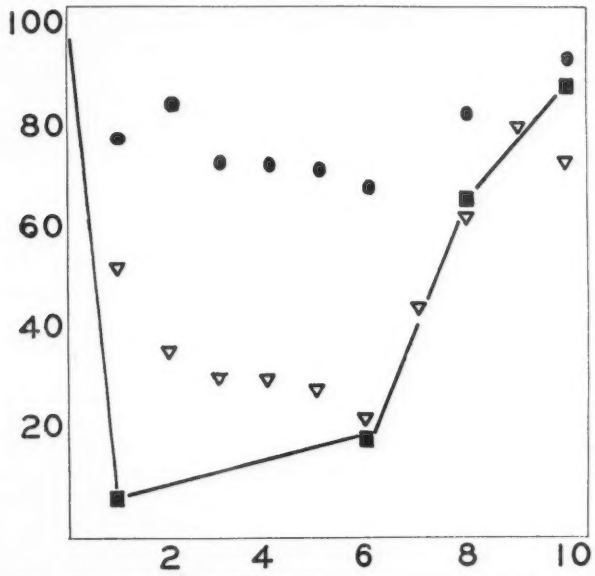


Fig. 1. THE GONADOTROPIC HORMONE(S) CONTENT, AND THE NUMBER OF EOSINOPHILS AND BASOPHILS IN THE RABBIT PITUITARY AT ESTRUS AND FOLLOWING STERILE COITUS. The abscissae indicate in days the time following coitus, during estrus. The ordinates indicate the number of cells (10) or the gonadotropic content of the rabbit pituitary in percentage of the levels found in the estrous gland. [Dots = eosinophils (10), triangles = basophils (10), solid squares = gonadotropic hormone (11).]

rabbit pituitary, the histological investigations of Wolfe et al., are of particular interest. In the 48 hours following coitus, these investigators found a very sharp decrease in the relative number of basophils in the rabbit gland, with very little change in the relative number of eosinophils. The temptation is great to correlate the precipitous post-coital decline of gonadotropic hormone with the almost parallel changes in the number of basophils (fig. 1), and to accept this correlation as further evidence in support of the thesis presented by Severinghaus.

Yet, there are some data which are not in accord with this thesis. The growth hormone first appears in the fetal pig pituitary (4) at a time when the chromophil cells are predominently basophilic (8). At a later stage of development, the first appearance of gonadotropic hormone is associated with a pronounced increase in the number of eosinophils. A recently described dwarf mutant of the Polish rabbit is characterized by a pituitary which is predominantly eosinophilic, the basophils being reduced in

number (9). Perhaps it is worth recalling that the dwarf mouse, whose pituitary is devoid of eosinophils (7) exhibits not only a failure of somatic growth, but also unmistakable deficiences in the organs of reproduction.

The future may enable us to reconcile these conflicting data. At the moment, however, it does not seem possible for us to identify the hormonal functions of the chromophil cells of the pituitary with any degree of surety. Certainly, our own data do not permit us to assign either prolactin or the gonadotropic hormone(s) to one or another of the cell types. To be sure, the highest concentration of gonadotropic hormone and the highest concentration of basophils are found in the medullary, rather than the cortical, area of the beef pituitary. But even in the medullary area the basophils may not exceed 50% of the total number of cells. By our present histological methods, the chromophobes and eosinophils of the medulla look quite like those of the cortex, but we cannot be sure that they are exactly alike. Therefore, we cannot exclude the possibility that the high gonadotropic hormone content, which distinguishes the medullary area, is attributable to the 50% (approximately) of the cells not accounted for by the basophils. Our experiments do indicate, however, that prolactin and the gonadotropic hormone(s) are not found in the same cell of the bovine pituitary gland.

SUMMARY

When tested by our methods, the beef pituitary shows a much higher concentration of gonadotropic hormone(s) in the medulla (central portion) than in the cortex (peripheral portion). The reverse is true of prolactin, but in this case the difference is not so great. It follows therefore, that the two hormones are probably not found in the same cell of the bovine pituitary. In all likellhood this indicates that the two hormones are formed by different cells.

During a time interval when the rabbit pituitary is almost completely exhausted of its content of gonadotropic hormone(s) there is no significant change in the prolactin content. Unless the rate of prolactin formation is so rapid as to maintain its initial concentration in the face of a high rate of discharge, we must conclude that prolactin and the gonadotropic hormone are secreted by the rabbit pituitary at independently variable rates.

Therefore, in both the bovine and the rabbit pituitary, prolactin and the gonadotropic hormone behave as separate and independent physiologic entities.

The additon of an extract of beef muscle to solutions containing prolactin augments the biologic effectiveness of the latter. It is therefore impossible to make a comparison of the prolactin activity of crude extracts with that of relatively purified preparations which contain much less inert material. Nevertheless, when the amount of inert matter is kept constant, variations in the concentration of 'prolactin' are easily detectably by the crop gland response if the differences are of the order of 30 to 40%.

REFERENCES

1. Riddle, O., and R. W. Bates: p. 1102, Sex and Internal Secretions, Williams & Wilkins, Baltimore, 1939.
2. Friedman, M. H.: *Endocrinology* 24: 617. 1939.
3. Smith, P. E., and I. Smith: *Anat. Rec.* 25: 150. 1923.
4. Smith, P. E., and Dortzbach: *Anat. Rec.* 43: 277. 1929.
5. Schooley, J. P., and O. Riddle: *Am. J. Anat.* 62: 313. 1938.
6. Hall, S. R.: *Anat. Rec. (supp.)* 72: 123. 1938.
7. Severinghaus, A. E.: Sex and Internal Secretions, p. 1045. Williams & Wilkins, Baltimore, 1939.
8. Nelson, W. O.: *Am. J. Anat.* 52: 307. 1933.
9. Greene, H.: *J. Exper. Med.* 71: 839. 1940.
10. Wolfe, J. M., D. Phelps and R. Cleveland: *Am. J. Anat.* 55: 363. 1934.
11. Friedman, M. H., and G. S. Friedman: *Endocrinology* 24: 626. 1939.

EFFECT OF LIGHT ON SEXUAL MATURATION, ESTROUS CYCLES, AND ANTERIOR PITUITARY OF THE RAT[1]

VIRGINIA MAYO FISKE

From the Harvard Biological Laboratories

CAMBRIDGE, MASSACHUSETTS

THE FACT that light stimulates many vertebrates, hastening the onset of the breeding season or inducing precocious sexual maturity, has already been established. It has also been shown that this stimulation of the gonads and accessories is mediated through the anterior pituitary. For a review of the subject see Bissonette (1).

The effect of light on a polyestrous mammal was investigated by Hemmingsen and Krarup (2), who found that the normal diurnal variations associated with estrus in the rat were reversed when the day-night rhythm of light was artificially shifted. They further reported a prolonged cornification of the vagina in rats kept under continuous light. Browman (3), confirmed the effect of light on the vaginal smear and also found no significant change in the age at which vaginal introitus occurred.

The purpose of this work was to study the effects of continuous illumination and darkness on the estrous cycle, the time at which vaginal introitus appeared, and the balance of the luteinizing and follicle stimulating hormones of the anterior pituitary.

Experimental conditions. The rats kept in the light were housed in wire cages illuminated from above by 100-watt bulbs equipped with reflectors. Measurement of the average illumination in the cages by means of a photometer showed that the intensity was about 70 foot-candles per square foot. The animals in darkness were similarly caged in a photographic darkroom, and all examinations were made in a faint green light or through the use of a substage lamp. The temperature of both rooms varied little from normal room temperature.

Effect of light on vaginal introitus. Rats kept in light, dark and under ordinary laboratory conditions from the twenty-first day of life were examined daily for vaginal introitus. Of the 150 rats so studied it was found that those kept in the light were the first to establish the vaginal orifice and those in the dark last (table 1). Similar results were obtained when the rats were kept in light or dark from birth, indicating that treatment earlier than the twenty-first day was ineffectual. Such a hastening in the opening of the vaginal membrane is generally known to result from the administration of the follicle stimulating hormone, (FSH), so the indications were that the pituitary, under the influence of light, had released larger amounts of this hormone. The very marked retardation in the maturation of the females kept in the dark might be explained as due either to a decrease or absence of FSH secretion as a result of the lack of light, or, to the inhibition of FSH as a result of a marked increase in the secretion of luteinizing hormone (LH) (4).

Effect of light on the sexual cycles of rats. As soon as the vaginal orifice of rats kept

Received for publication May 26, 1941.

[1] Supported in part by a grant from the Rockefeller Foundation administered by Professor Frederick L. Hisaw.

TABLE 1. COMPARISON OF THE AGE AT WHICH THE VAGINAL MEMBRANE RUPTURES IN RATS KEPT UNDER CONSTANT ILLUMINATION, LABORATORY CONDITIONS, AND IN DARKNESS (50 ANIMALS IN EACH GROUP)

Age in days	Number mature (%)		
	Light	Laboratory	Dark
39	8	0	0
41	20	2	0
43	34	2	0
45	50	5	0
47	76	28	4
49	82	40	4
51	90	50	15
53	98	60	15
55	100	70	21
57		81	21
59		83	40
61		85	50
63		87	55
65		90	60
67		100	65
69			70
71			77
73			81
75			83
77			90
79			91
81			96
89			98
90			100

in light or darkness was found to have been established they were examined daily by means of vaginal smears for changes in the estrous cycle. Rats kept in the light were found to have prolonged periods of estrus, which is in agreement with the results of Hemmingsen and Krarup (2), and Browman, (3). Light-treated rats also exhibited somewhat lengthened periods of diestrus, whereas animals kept in the dark were continually fluctuating between estrus and diestrus and showed longer periods of metestrus than did the normal rat, (table 2). Browman's work on rats kept in the dark indicated no change in the cycle, but the rats used by him were fully mature before they were placed in the dark and therefore were possibly less susceptible to changed light conditions.

Long continued injection of estrogens into spayed female rats has been shown to

TABLE 2. DURATION AND NUMBER OF PERIODS OF ESTROUS (E), DIESTROUS (D), AND METESTROUS (M) SMEAR EXHIBITED BY 50 RATS KEPT IN THE LIGHT OR DARK OVER A 60-DAY INTERVAL

Length of period, days	Dark room			Light room		
	E	D	M	E	D	M
1	100	99	112	31	47	76
2	63	40	46	31	39	7
3	22	15	23	47	28	3
4	11	6	9	31	17	0
5	2	3	4	22	19	1
6	1	3		2	8	
7		2		4	6	
8				1	5	
9				1	5	
10				1	1	
37				1		

TABLE 3. OVARIAN AND UTERINE WEIGHTS OF IMMATURE FEMALE RATS KEPT IN LIGHT OR DARKNESS FOR 15–20 DAYS AND INJECTED WITH PU OR FSH (APPROX. 1 R.U.)[1]

Treatment	Series I		Series II		Series III
	Ov. wt.	Ut. wt.	Ov. wt.	Ut. wt.	Ov. wt.
Light controls	12.5	20	14.0	23	
Dark controls	13.0	23	13.5	22	
Light and PU	23.0	137	19.0	109	32.0
Dark and PU	18.0	90	15.0	68	23.0
Light and FSH	24.0	63	16.0	41	27.0
Dark and FSH	32.0	101	22.0	71	45.0

[1] Every figure is the average of 5–10 determinations.

result in prolonged periods of estrus (5). Since the secretion of estrogen by the ovaries has been found to be dependent upon FSH (6), the lengthened estrous periods, characteristic of rats kept in the light, are evidence of the secretion of FSH.

Tests for FSH and LH in immature male and female rats kept in the light or darkness for 5, 10, 15, or 20 days. It has been shown that the action of FSH markedly augments the action of the hormone from pregnancy urine (PU) (7), and the greater the amount of FSH present, the greater the response obtained. With this in mind the amount of FSH secreted by female rats kept in the light or in the dark for 5, 10, 15, or 20 days was tested by injecting all the animals with a constant amount of PU. The animals were 21 or 22 days old when light treatment was instituted. Injections were made twice daily during the last 4 days of light treatment. The animals were killed on the morning following the last injections and the ovaries and uteri weighed.

No difference in response was detected until the animals had been in the light or dark for 15 days. In both the 15 and 20-day series the ovaries and uteri of the females kept in the light and injected with PU during the last 4 days were definitely heavier than the ovaries and uteri of the animals kept in darkness and given the same treatment, (table 3), indicating that more FSH was being released in the females kept in the light than in those kept in darkness.

A similar study was made on sexually immature male rats that had been kept in light or darkness for 15 or 20 days. They were given PU, as described for the females, and the weights of the seminal vesicles compared. This procedure was based on the fact that FSH alone does not affect the size of the seminal vesicles but does increase

TABLE 4. TESTICULAR AND SEMINAL VESICLE WEIGHTS OF MALES KEPT IN LIGHT OR DARKNESS FOR 15–20 DAYS AND INJECTED WITH PU OR FSH (APPROX. 1 R.U.)[1]

Treatment	Series I		Series II		Series III	
	Testes wt.	Sem. ves. wt.	Testes wt.	Sem. ves. wt.	Testes wt.	Sem. ves. wt.
Uninjected light control	502	13.0	583	14.5	555	13.5
Uninjected dark control	326	9.0	447	11.0	436	9.5
Light and PU	747	22.0	787	20.0	1015	45.0
Dark and PU	343	13.0	434	16.0	432	22.0
Light and FSH	988	16.5	590	16.0	831	20.0
Dark and FSH	300	9.0	451	11.5	356	11.0

[1] Every figure is the average of 5–10 determinations.

the secretion of androgens when given simultaneously with a luteinizing factor such as PU or LH (8). In the males, as in the females, a greater amount of FSH seemed to be present in the animals kept in the light, for their seminal vesicles were conspicuously enlarged by the PU injections while those of the males in the dark were not (table 4).

In an effort to measure the amount of LH being secreted in light treated rats as compared with those in darkness use was made of the augmentation of FSH by LH. Fevold, Hisaw and Leonard (9) have shown that the effect of FSH on the immature rat ovary is very much greater if LH is also present and the degree of augmentation is an indication of the amount of LH present (10). With these facts in mind, females were kept in light or darkness 5, 10, 15, or 20 days and were injected with FSH the last 4 days. Under these circumstances the ovarian and uterine weights of females in the dark for 15 or 20 days were heavier than those in the light (table 3) indicating a greater secretion of LH in the former than in the latter.

The FSH-LH augmentation reaction on seminal vesicle growth previously described was used in assaying the amount of LH secreted by immature males kept in light or darkness for 15 or 20 days. In contrast with the situation which obtains in females, the males apparently secreted no more LH in the dark than in the light. In fact, the seminal vesicles of males kept in the light were somewhat larger than those of the dark room males both prior to and following the FSH injections (table 4).

Study of the relative seminal vesicle and ovarian growth potency of pituitaries of males and females kept in the light and dark. It has often been pointed out that an analysis of the pituitary of an animal may be no indication of the actual activity of the gland since it measures only the amount of hormone present at the time of extraction. However, it seemed worthwhile to proceed further and test the pituitaries from both normal and castrated males and females kept in the light or dark for 8 weeks. Castration and light treatment were instituted on the twenty-first day of life so the rats were 77 days old when killed.

The pituitary material was ground to a fine powder in acetone, dried and taken up in water to be injected either on the basis of equal weight or in pituitary gland equivalents. Investigations on the effectiveness of the subcutaneous versus the intraperitoneal method of injection of FSH and LH have shown that whereas the LH is equally effective when given in either manner, the FSH in otherwise adequate doses is unable to act in the rat when administered intraperitoneally (11). Therefore, it was possible to inactivate the FSH by injecting the unfractionated pituitary material intraperitoneally and so measure only the LH. Intraperitoneal injections to measure LH cannot be used in the female since LH in the absence of FSH does not produce significant growth of an immature ovary (19). However, LH does largely control the development of the seminal vesicles through its stimulation of the interstitial tissue of the testes and the consequent release of male sex hormone (8). In other words, the relative amounts of LH present in an anterior pituitary extract may be determined by weighing the seminal vesicles of immature rats following intraperitoneal injections. There is no quantitative method for measuring the FSH in the presence of LH but since FSH is the controlling factor in producing ovarian growth in immature females this does serve as a rough measure of FSH (13).

Accordingly, the recipients, 21-day-old male or female rats, were injected twice daily for a period of 4 days with the pituitary preparations. They were killed on the morning of the fifth day and the ovaries and uteri or seminal vesicles and testes were weighed. The females were injected subcutaneously, the males intraperitoneally, and there were at least 75 pituitary donors in each of the groups tested. A comparison of the figures in table 5 indicates that the pituitary material from normal females or

TABLE 5. WEIGHTS OF THE OVARIES AND UTERI, OR TESTES AND SEMINAL VESICLES OF FEMALE AND MALE RECIPIENTS FOLLOWING THE INJECTION OF THE EQUIVALENT OF THREE PITUITARIES FROM NORMAL AND CASTRATED MALE AND FEMALE RATS KEPT IN THE LIGHT OR DARK FOR 56 DAYS

Donors	Recipients[1]			
	Ovarian wt.	Uterine wt.	Testicular wt.	Seminal vesicle wt.
Norm. male Dark	53	87	408	15
Norm. male Light	79	84	451	13
Cast. male Dark	64	73	414	18
Cast. male Light	88	81	420	16
Norm. female Dark	19	105	436	17
Norm. female Light	25	94	407	12
Cast. female Dark	50	58	423	18
Cast. female Light	61	54	414	14
Uninjected controls	12	24	409	9

[1] At least 20 recipients in each group.

males kept in the light was more effective in inducing ovarian development than was such material from normal females and males kept in the dark. Moreover, pituitaries from females in the dark were more potent in stimulating seminal vesicle growth than pituitaries from female light room donors. It is clear then, that the pituitaries of females kept in the dark are richer in LH than those of the females in the light. On the other hand, since ovarian weight is the result of the combined action of FSH and LH and since we have shown that the amount of LH present has been reduced in the pituitaries of the females kept in the light, the greater ovarian stimulating action of such glands must be due to an increased FSH content.

Unlike the females, the males showed no significant difference in the amount of luteinizing hormone in pituitaries from light and dark room donors as judged by the increase in seminal vesicle weight. Since the amount of the LH was no greater in

TABLE 6. WEIGHTS OF THE OVARIES AND SEMINAL VESICLES OF FEMALE AND MALE RECIPIENTS FOLLOWING THE INJECTION OF PITUITARY POWDER FROM NORMAL MALE AND FEMALE RATS KEPT IN THE LIGHT OR DARK FOR 100 TO 300 DAYS

Donors	Dose	Length of treatment	Recipients[1]	
			Ovarian wt.	Sem. ves. wt.
	mg.	days	mg.	mg.
Females				
light	4	100	25 (3)	20 (4)
dark	4	100	12 (4)	25 (4)
light	3	250–300	15 (5)	12 (5)
dark	3	250–300	21 (5)	18 (5)
Males				
light	3	250–300	15 (5)	10 (5)
dark	3	250–300	17 (5)	14 (5)
Controls			12	9

[1] Figures in parenthesis indicate number of animals injected.

males kept in the light as compared with those in the dark, the added ovarian growth induced by their pituitaries must have been due to an increased release of FSH.

Pituitaries from castrated rats kept under the same conditions as those just described for normal rats gave results which were similar in respect to FSH-LH balance. Castration did, however, bring about an increase in the amount of both FSH and LH in all groups (table 5).

Pituitaries from older female rats that had been exposed to light or kept in the darkroom continuously for 100 days were also tested. Here again, the pituitaries of those exposed to light were found less effective for stimulating growth of the seminal vesicles and more active for increasing ovarian weight than the pituitaries of similar rats kept in the dark (table 6). However, after a period of 250 to 300 days in the dark

TABLE 7. WEIGHTS OF THE PITUITARY, UTERUS, AND OVARIES IN FEMALE RATS PLACED IN THE LIGHT, THE DARK, AND UNDER LABORATORY CONDITIONS WHEN 3 WEEKS OLD AND KEPT THERE UNTIL 77 TO 350 DAYS OF AGE

Age in days	No. of rats	Pituitary wt.	Uterine wt.	Ovarian wt.
		mg.	mg.	mg.
		Light		
Nf[1] 77	15	6.9	216	35
Cf 77	25	8.6		
Nf 100	38	9.5		43
Nf 200	19	10.0	345	26
Nf 300	22	9.3	471	32
		Laboratory		
Nf 77	10	6.1	61	16
Cf 77	10	8.1		
Nf 200	10	9.6	420	50
		Dark		
Nf 77	28	4.0	35	11
Cf 77	20	5.7		
Nf 100	24	10.7		41
Nf 200	20	10.0	513	55
Nf 300 to 350	14	10.7	579	50

[1] Nf represents normal females
Cf represents castrated females

or light dried pituitary powder from females kept in darkness is more effective than such material from animals kept in the light, in inducing both ovarian and seminal vesicle growth. It seems possible to explain this reversal in potency in one of two ways. Either the glands become partially exhausted after so long a period in the light, or the animals in the lights wing so far toward the production of FSH that there is too little LH in their pituitaries to cause either an increase in seminal vesicle growth or augmentation of the action of FSH on the ovary.

Examination of table 6 will also show no significant difference in the amount of FSH or LH in equal weights of pituitary powder from males kept in light or in darkness for 250 to 300 days. The test animals did show a slight difference in ovarian and seminal vesicle weights which is not significant since the pituitaries of the males in the dark were smaller than those of males in the light (table 8). Here again it is interesting to note that, as in the females, the increased amount of FSH found in the pituitaries of males exposed to light for 56 days is not found when treatment is prolonged for 250 to 300 days.

Directly observable differences found in rats kept in the light or dark. Perhaps the

strongest evidence bearing on FSH-LH balance in rats kept in light or darkness comes a study of the directly measurable morphological changes found in the two groups. Weights were taken of the body, thyroid, adrenal, pituitary, ovaries, testes, uterus and seminal vesicles of over 250 rats placed in continuous light or darkness when 21 days of age and killed when 77, 110, 150, 200, 250, 300 or 350 days old. No striking differences were found in the weights of the body, thyroid and adrenals so these data will not be presented. By 77 days of age, after 56 days of constant illumination or darkness, the pituitaries of both males and females kept in the dark were definitely smaller than those of the animals kept in the light, and slightly smaller than the pituitaries of rats kept under laboratory conditions (tables 7, 8). The ovaries of the light treated females were much larger than those of the females kept in darkness, and

TABLE 8. WEIGHTS OF THE PITUITARY, SEMINAL VESICLES, AND TESTES IN MALE RATS PLACED IN THE LIGHT, THE DARK, AND UNDER LABORATORY CONDITIONS WHEN 3 WEEKS OLD AND KEPT THERE UNTIL 77 TO 250 DAYS OF AGE

Age in days	No. of rats	Pituitary wt.	Sem. ves. wt.	Testes wt.
		mg.	*mg.*	*gm.*
		Light		
Nm[1] 77	15	4.6	204	1.83
Cm 77	17	8.6		
Nm 100	18	5.3	652	2.50
Nm 150	10	11.3	997	3.09
Nm 200	12	8.6	890	2.90
Nm 250	5	8.8	1,039	3.28
		Laboratory		
Nm 77	10	4.2	188	1.72
Cm 77	10	8.4		
Nm 150	10	8.4	1,036	3.07
Nm 250	10	8.5	1,230	3.17
		Dark		
Nm 77	15	3.5	88	1.18
Cm 77	21	7.0		
Nm 100	12	4.8	492	2.36
Nm 150	10	5.5	274	2.02
Nm 250	18	5.8	849	2.81

[1] Nm represents normal males
Cm represents castrated males

somewhat larger than those of rats kept under normal laboratory conditions (table 7). The greater weight of the ovaries of the light treated animals may have been due to the fact that the pituitaries of such animals were heavier, contained larger amounts of FSH, and probably secreted larger amounts of this hormone than the pituitaries of the females kept in darkness.

Histological studies showed that the ovaries of females in the light were functioning as they contained both follicles and corpora lutea while the ovaries of females kept in darkness were for the most part infantile. The uteri reflected the ovarian conditions. Those of females having undeveloped ovaries were small and the uterine mucosa resembled that of an immature animal. The uteri of the females kept in the light were much larger and the uterine epithelium in about 90% of the cases was the unusually tall columnar type characteristic of an estrogen response. The fact that so large a proportion of the light treated animals showed the estrogen type of uterus was a further indication of FSH release since this hormone stimulates secretion of estrogens by the ovary.

The pituitaries of the light or dark treated females at 110 to 300 days of age did not vary significantly in weight (table 8) although the gonad stimulating ability did decrease in females exposed to light for as long as 250 days (table 6).

The ovaries of females kept in the light or darkness for 110 days weighed the same and exhibited both follicles and corpora lutea but as the period of exposure to light was lengthened the ovaries became increasingly follicular and regressed in size (table 7). By 200 days of age the average weight of the ovaries of females in the light was only 26 mg. while those in darkness were almost twice as heavy. Furthermore, the ovaries of the darkroom females appeared fully functional and contained many corpora lutea while at least 90% of the ovaries of rats kept in the light showed no corpora and contained only a few atretic follicles. The absence of corpora lutea was interpreted as an indication of a lack or subthreshold secretion of LH from the pituitary. Further support for such an explanation was found in the histology of the regressing ovaries, as the interstitial tissue consisted largely of 'deficiency' or 'wheel' cells (12). These cells occur in large numbers following hypophysectomy and may be restored to a normal appearance by the injection of the luteinizing factor of the pituitary (13). Therefore it is reasonable to conclude that the presence of 'deficiency' cells indicates a lack of LH in the organism.

The uteri of fully mature females in the light or darkness for 30 to 350 days continue to show all stages of the estrous cycle but one-third of those in the light for as long as 350 days showed signs of metaplasia or pyometra. Apparently metaplasia, induced by the release of large amounts of estrogens, was maintained by their chronic secretion. Only one instance of slight inflammation was found in the females of the same age kept in darkness for the same length of time. Metaplasia of the uterus was evident in the transformation of the simple columnar epithelium to stratified squamous epithelium. Animals having pyometra frequently showed marked enlargement of the uterus and salpingitis. The lumena of such uteri were packed with necrotic tissue and leucocytes and the wall heavily infiltrated. Metaplasia of the uterus or cervix has been induced in monkeys, rats and in guinea pigs by chronic injections of estrin (14). Pyometra or inflammation of the uterus of rats has also been reported following a continued injection of estrogen (15) so the high incidence of metaplasia and pyometra are further evidence of continued secretion of estrogen in the light treated animals.

The seminal vesicles and testes of the 77-day-old males kept in the light for 8 weeks were heavier than those of males in darkness (table 8). It has already been shown that the pituitaries of light treated males were the larger and contained more FSH so the greater growth of the testes was probably due to an increased secretion of this hormone. Seminal vesicle growth though induced primarily by LH is very much greater if FSH is also present (8). Therefore, the large size of the seminal vesicles of light treated males was perhaps due to the action of LH augmented by a large amount of FSH. The small seminal vesicles of the males kept in darkness might have been the result of a greatly decreased secretion of FSH and consequent lack of LH augmentation. The testes showed no obvious histological differences [other than more active spermatogenesis] in the light treated animals.

Males kept in light or darkness as long as 150 days showed very marked differences in pituitary, seminal vesicle and testicular weights. Testes of light treated males of this age were also found to be undergoing more active spermatogenesis and contained larger tubules than males in darkness. The greater spermatogenesis in the light treated males after 77 and 150 days treatment further indicated greater FSH secretion in that group (8).

By 250 days of age the pituitaries of the light treated males had regressed a little

in size and those of the males in darkness had increased but were still smaller than those of males kept in the light. Concomitant with the pituitary increase in the males kept in the dark the seminal vesicles and testes increased in weight, lending further strength to the suggestion that the small size of these organs in 77 and 150-day-old males was due to a deficiency in the amount of gonadotropic hormone being released.

SUMMARY AND CONCLUSIONS

Immature female rats kept in the light from birth or from the twenty-first day of life came into sexual maturity, as evidenced by the age at which vaginal introitus occurred, a little earlier (6 days) than females kept under normal laboratory conditions, and much earlier (16 days) than females kept in darkness. Since the opening of the vaginal orifice is dependent upon estrogen which is secreted under FSH stimulation, these results indicate increased secretion of FSH at an earlier age in the rats kept in the light.

The estrous cycles of young females kept in the dark or light were modified, as indicated by the cyclic changes in the vaginal smears. Those in the light exhibited unusually long periods of estrus and slightly lengthened periods of diestrus, while those in darkness were conspicuous for the number of days on which they showed metestrus smears. These results again point to a release of FSH in the females in the light and a shift in the balance of FSH and LH in females in darkness.

The injection of PU into males and females kept in the light 15 to 20 days gave better ovarian and seminal vesicle development than in animals living in darkness. This was interpreted as indicating that in the animals in the light there was more FSH to be augmented by the PU.

The injection of FSH into immature females kept in the light 15 to 20 days caused less ovarian growth than did an equivalent amount of FSH injected into females kept in darkness. This was interpreted as indicating that in females in the dark there was more LH being secreted to augment the FSH injected. Males similarly injected showed no such increase in LH release in the dark as the seminal vesicles of males in the light were heavier than those in the dark.

Pituitaries of 77-day-old males and 77 and 100-day-old females put in the light at 21 days of age were richer in FSH than were pituitaries from animals kept in darkness as tested by the ability of such material to induce ovarian growth. Under similar circumstances, the pituitaries of females in the light contained less LH than the pituitaries of females in the dark as tested by their ability to cause seminal vesicle growth. On the other hand, pituitaries from males similarly treated and tested showed no significant variation in LH secretion.

Tests of the gonadotropic potency of the pituitaries of 77-day-old male and female castrates indicated that they were affected by light in the same way as the normal animals in respect to FSH-LH balance but both hormones were secreted in larger amounts.

Pituitaries of female rats left in the light for as long as 250 to 300 days were less effective than those of animals of the same age kept in darkness in causing ovarian growth, thus indicating a shift in FSH content for shorter light stimulation produced the opposite result. Pituitaries from males similarly treated no longer showed the variation in FSH-LH content which was found after 8 weeks of light treatment.

Pituitaries, ovaries, and uteri of 77-day-old female rats kept in the light for 8 weeks were larger than those of females kept in darkness. The uterine epithelium of the light treated females was, in 90% of the cases, of the tall columnar type indicative of estrogen secretion.

Females, 21 days old, placed in light or in darkness until 110 days of age no longer

exhibit any difference in respect to the size of their uteri, ovaries, or pituitaries. Although their pituitaries remain the same size for the next 150 to 200 days, the ovaries of 250-day-old females in the light show fewer corpora lutea and have decreased in weight. By 300 days of age many of the females in the light appear to have ovaries which are functioning at a low level indicating secretory exhaustion of the pituitary or premature senility of the ovary due to overstimulation.

Ovaries of females exposed to the light 350 days exhibited large numbers of deficiency cells which are believed to point to a lack of the luteinizing hormone.

Females exposed to light for as long as 350 days frequently showed metaplasia of the uterus and secondary pyometra. Both these phenomena have been induced experimentally with long continued injections of estrogen and their occurrence may be considered further evidence of estrogen secretion.

The pituitaries, testes and seminal vesicles of males kept in the light for 77 to 250 days are larger than those of the animals in darkness. The difference in size is greatest when the rats are about 150 days of age. After this time the organs of those in the dark gradually increase approaching in size those of the males in the light. The increased weight of the testes of males kept in the light was due to the enlarged tubules in which active spermatogenesis was more prevalent than in the testes of males kept in darkness. There was some evidence of a slight increase in the proportion of interstitial tissue to tubular tissue in males kept in darkness but since their testes were so much smaller than those of animals in the light a comparison is impossible with the data at hand. These facts again point to a greater release of FSH under exposure to light.

The results which have just been presented make evident the fact that stimulation of the pituitary of the male or female rat by light increases the production and release of FSH. Conversely, a lack of light favors a decrease in FSH and, in the female only, an increase in LH secretion. That light may be a factor in controlling pituitary activity in the rat and therefore the estrous cycle is clear when one considers that normally the release of FSH results in an increased release of estrogen which in turn causes a decrease in FSH secretion and an increase in the release of LH. Yet under the influence of light stimulation female rats may stay in an estrous condition for weeks, indicating that the effect of light in causing FSH release is stronger for a time than the effect of estrogen on the pituitary.

It is a pleasure to acknowledge here the help freely given by Dr. H. L. Fevold during the course of this investigation.

REFERENCES

1. Bissonette, T. H.: Chap. XIX in the Pituitary Gland, Williams & Wilkins Co., Baltimore, 1938.
2. Hemmingsen, A. M., and N. B. Krarup: *Det. Kgl. Danske Videnskabernes Selskab* 13: 5. 1937.
3. Browman, L. G.: *J. Exper. Zool.* 75: 375. 1937.
4. Fevold, H. L., and V. M. Fiske: *Endocrinology* 24: 823. 1939.
5. Allen, E., F. L. Hisaw and W. U. Gardner: Chap. VIII in Allen, E.: Sex and Internal Secretions, Williams & Wilkins Co., Baltimore, 1939.
6. Fevold, H. L.: *Endocrinology* 28: 33. 1941.
7. Evans, H. M., K. Meyer and M. E. Simpson: *Am. J. Physiol.* 100: 141. 1932.
8. Greep, R. O., H. L. Fevold and F. L. Hisaw: *Anat. Rec.* 65: 261. 1936.
9. Fevold, H. L. F. L. Hisaw and S. L. Leonard: *Am. J. Physiol.* 97: 291. 1931.
10. Fevold, H. L., F. L. Hisaw and R. O. Greep: *Am. J. Physiol.* 114: 508. 1936.
11. Fevold, H. L.: *J. Biol. Chem.* 128: 82. 1939.
12. Selye, H., J. B. Collip and D. L. Thomson: *Endocrinology* 17: 494. 1933.
13. Evans, H. M., K. Korpi, M. E. Simpson, R. I. Pencharz and D. H. Wonder: *Univ. Cal. Pub. Anat.* 1: 255. 1936.
14. McEuen, C. S., H. Selye and J. B. Collip: *Lancet* 230: 775. 1936.
15. Kaufmann, C., and E. Steinkamm: *Arch. F. Gynäk.* 162: 553. 1936.

THE STRUCTURE OF BOWMAN'S CAPSULE IN CASTRATE AND TESTOSTERONE TREATED MALE MICE AS AN INDEX OF HORMONAL EFFECTS ON THE RENAL CORTEX[1]

CHARLOTTE E. CRABTREE
From the Department of Zoology, Smith College
NORTHAMPTON, MASSACHUSETTS

IN THE KIDNEY of a few species of mammals, including mice, a type of Bowman's capsule may occur in which the squamous cells usually constituting the parietal lamina are partially or completely replaced by cuboidal cells resembling those making up the walls of the proximal convoluted tubule. A method has recently been described (1) for determining, by means of a differential count, the relative number of this cuboidal-cell type of capsule, and a number of age and sex correlations in the postnatal development of the mouse kidney have been demonstrated. A few cuboidal or 'high' cell capsules are present in both sexes at birth; the percentage of cuboidal cell capsules increases in both sexes for the first 20 weeks of postnatal life, but at all times after puberty the relative number of cuboidal cell capsules in the male mouse kidney far exceeds that in the female; this sexual variation in the kidney occurs at puberty and is closely correlated with the time of degeneration of the androgenic or x-zone of the mouse adrenal gland (2).

These sexual variations in the structure of Bowman's capsule—first evinced at the onset of sexual maturity and closely correlated with the development of the adrenals—strongly suggest an endocrine factor or factors as causes of measurable histological changes in the renal cortex of the mouse. This study was made to estimate the effects on the renal cortex of castration and treatment of castrates with testosterone propionate.

It has been known for some time that generalized renal hypertrophy follows injections of androgens and estrogens. Working with reptiles, Noble and Greenburg (3) noted that a remarkable hypertrophy of certain tubules of male *Anolis* resulted from injections of testosterone propionate; they also stated that Kehl obtained similar results with androsterone benzoate in the adult female *Uromastix*. In castrated rats a smaller than normal renal weight exists and a significantly greater renal weight results in castrates treated with an impure testosterone, androsterone and estrone. Renal weights were normal in castrates which had received injections of testosterone for long periods of time (4, 5, 6).

More recently Selye (7) reported marked enlargement and a 42% increase in renal weight in female mice treated with large daily doses of testosterone propionate. Pfeiffer, Emmel and Gardner (8) used young male mice as experimental animals and extended Selye's work to include the effects of estradiol benzoate, estradiol dipropionate and testosterone. Hypertrophy was observed in all cases of treated animals, but the greatest degree occurred when estrogens and androgens were administered

Received for publication April 18, 1941.

[1] Contributions from the Department of Zoology, Smith College, No. 193. A thesis submitted to the faculty of Smith College in partial fulfilment of the requirements for the degree of Master of Arts.

simultaneously. In addition to a generalized hypertrophy of the renal tubules, these workers commented on the presence of cuboidal cell capsules in the kidneys of both the experimental animals and the controls, but noted them most frequently in the kidneys of animals receiving hormone injections, particularly of testosterone propionate. Ludden, Kreuger and Wright (9) repeated the work of Selye on rats, and observed a generalized enlargement of the tubules in all the animals treated, but noted no other deviation from the normal morphology in either the experimental animals or the controls.

The differential count of the two types of Bowman's capsules has proven to be a reliable and sensitive index of age and sex variations in normal mice, and is conceivably regulated by hormonal control. Using this method for measurement, the following study was made to determine the effects of castration and treatment with testosterone propionate on the mouse kidney.

MATERIAL AND METHODS

The mice used in the following experiment were from two strains. One group of 12 animals was albinos from 2 litters born 3 days apart, and with the same paternal parent. The second group of 6 animals was from the hybrid stock of the Smith College animal colony. Of the first group, 8 males of 3 to 4 weeks of age were castrated under ether anesthesia by direct incision into the scrotal sac, and one normal male (*D25*) and one normal female (*D24*) were killed for controls. Following a 5-week recovery period one of the 8 castrates (*D35*) was killed, and injections were started on 5 of the remaining 7. Testosterone propionate (25 mg. per 1 cc. of sesame oil)[2] was given subcutaneously to the 5 animals in the following dosages: 2 received 2.5 mg. daily in a single dose, one 5 mg. daily in a single dose, and two 10 mg. daily in a divided dose. The 2 remaining castrates were kept untreated. At the end of a 3-week injection period, when the animals were 10 to 11 weeks old, the entire group was killed, together with a normal, untreated male and female of the same age.

Of the second group, two males (*D40* and *D41*) were castrated with the same technic at 5 weeks of age, and a normal male (*D12*) and female (*D13*) 3 days younger were killed for controls. Following a 5-week recovery period *D41* was injected daily for 3 weeks with 5 mg. of testosterone propionate, and *D40* was kept untreated. *D7* ♀ and *D11* ♂ were killed at 11 weeks and 2 days of age as normal controls, and *D40* and *D41* killed at the end of the injection period when they were 13 weeks old.

The animals were killed by a blow on the head, the kidneys were removed, fixed in Heidenhain's fixative, dehydrated in dioxan, embedded in paraffin, and sectioned at 6μ. Both kidneys and adrenals were weighed (correct to the nearest mg.) 24 hours after the animals were killed. One kidney of each animal was cut transversely and one longitudinally; 3 sections of each kidney were stained with Mallory's triple stain and 3 with iron hematoxylin and eosin, making 12 sections in all.

Total body weights were determined as live weights, and intestinal contents disregarded. Thus the computed kidney weight:body weight ratios included are only approximate.

All the capsules on each slide were counted and classified as either the cuboidal or 'high' cell type or as the squamous or 'low' cell type. Capsules were regarded as 'high' if any cuboidal cells could be seen within the capsule. Since it is possible to section a capsule in which the parietal lamina is only partly composed of cuboidal cells without including any of these cells in the section, the figures quoted may be somewhat lower than the actual condition existing.

[2] The testosterone propionate used in these experiments was supplied by the Schering Corporation, Bloomfield, New Jersey, through the courtesy of Dr. Erwin Schwenk.

RESULTS

Table 1 is a summary of the results of the differential counts of the two types of Bowman's capsules correlated with various data concerning the weights of the organs and the condition of the androgenic zones of the adrenals.

TABLE 1. RESULTS OF THE EFFECT ON THE KIDNEY OF CASTRATION AND TREATMENT WITH TESTOSTERONE PROPIONATE

	Series No.	Age in wk.-days	Dose T.P.	Kidney weight	Kid. wt.: body wt.	% High cap.	% Low cap.	Condition of the androgenic zone
Control group	*D24*♀	2-6	—	0.193	1.3	16	84	—
	D25♂	2-6	—	0.177	1.5	8	92	—
	**D13*♀	4-3	—	0.088	1.6	29	71	—
	**D12*♂	4-3	—	0.083	1.7	37	63	—
	D26♀	10-6	—	0.363	1.6	30	70	Well developed
	D27♂	10-6	—	0.383	1.5	80	20	Degenerated
	**D7* ♀	11-2	—	—	—	68	32	—
	**D11*♂	11-2	—	—	—	84	16	—
Untreated castrates	*D35*♂	8-4	0	0.318	1.2	22	78	—
	D32♂	10-0	0	0.382	1.3	32	68	Well developed
	D39♂	11-2	0	0.443	1.4	22	78	Well developed
	**D40*	13-0	0	0.347	1.5	23	77	—
Treated castrates	*D34*♂	10-0	5	0.688	2.4	70	30	Degenerated
	D33♂	10-0	10	0.613	2.0	59	41	Degenerated
	D36♂	11-2	2.5	0.683	2.1	77	23	Degenerated
	D38♂	11-2	2.5	0.597	1.9	69	31	Degenerated
	D37♂	11-2	10	0.612	1.9	62	38	Degenerated
	**D41*♂	13-0	5	0.687	4.4	76	24	—

* Older hybrid group from the Smith College animal colony. The remainder are younger albinos from Carworth Farm stock.

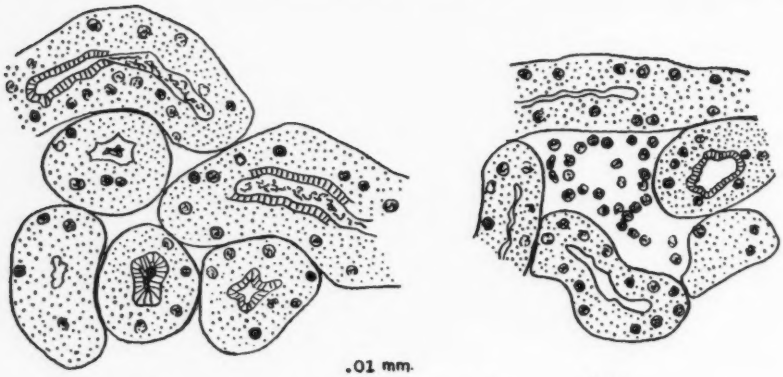
Doses of testosterone propionate are reported in mg. per 24 hours.

All kidneys of the albino stock were weighed with the adrenals adherent. The latter are so small as to effect only the fourth decimal place.

A. *Effect of castration.* As seen in the above table, castration affects the mouse kidney by *a*) decreasing slightly the kidney weight:body weight ratio, and *b*) by limiting the development of cuboidal cell capsules. In both the weight and the differential count the kidney of the castrate resembles that of an immature male or of a female of the same age.

The average absolute weight of the untreated castrates' kidneys is 0.413 gm. as compared with 0.383 gm. for the control animal, but this difference is far too small to be significant. The average kidney weight:body weight ratio, however, is 1.35% as compared with 1.5%, figures which are in accordance with the values obtained by Korenchevsky and Dennison in rats (5).

The condition of Bowman's capsule is a far more reliable means of differentiation between the kidney of the castrate mouse and the normal kidney. During the course of this experiment (in the group of animals from 3 to 11 weeks of age) the percentage of high cell capsules of the normal female was 16% at 3 weeks of age and 30% at 11 weeks. During the same interval in the life of the normal male a change from 8% at 3 weeks of age to 80% at 11 weeks occurred. In the two untreated castrates of the same age the change was from 8% to an average of 27%. In the older group (from 5 to 13 weeks of age) the rise in the percentage of cuboidal cell capsules in the normal female was from 29% at 4½ weeks to 68% at 11½ weeks, and during the same period in the normal male the percentage changed from 37% to 84%, while the kidney of the untreated castrate of the same age as the latter contained only 23% of cuboidal cell



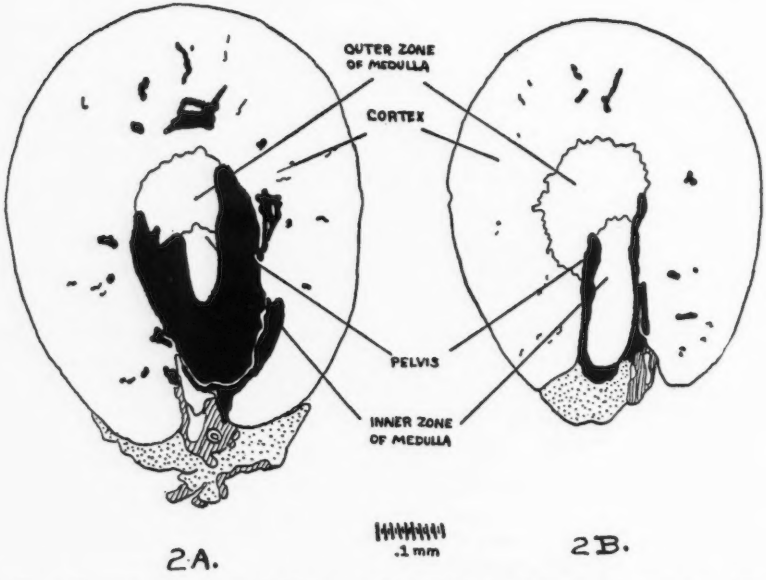


Fig. 1. CAMERA LUCIDA DRAWINGS of a section of the renal cortex of *a*) a castrate mouse given daily injections of testosterone propionate for 3 weeks and *b*) an untreated castrate of the same age.

Fig. 2. PROJECTION MICROSCOPE DRAWINGS of a cross section through the entire kidney of *a*) a castrate mouse given daily injections of testosterone propionate for 3 weeks and *b*) an untreated castrate of the same age.

capsules. Since the 4½-week-old kidney is immature and new cuboidal cell capsules may still be forming, this drop in the percentage of cuboidal cell capsules is not a priori evidence that dedifferentiation has occurred, but certainly few, if any, new high cell capsules formed during this period.

The results indicate that castration retards the development of cuboidal cell capsules in the male mouse kidney and results in a condition more nearly comparable

to that of the immature male or to the female of the same age. There was no marked evidence of castration effects on the cytology of the uriniferous tubules; the cells were normal in appearance and the lumina free from debris.

B. Effect of testosterone propionate. Treatment of castrate mice with testosterone propionate results in *a*) a marked renal weight increase aacompanied by *b*) hydronephrosis and *c*) an increase in the percentage of cuboidal cell capsules toward approximately the value manifested by the normal male.

The average absolute weight of the kidney of the testosterone treated, 10- to 11-week-old castrates was 0.639 gm., an increase of 66% over that of the normal male and 54% over the average renal weight of the 2 untreated castrates. The kidney weight:body weight ratio of the same group of treated castrates averaged 2.1% as compared with 1.5% for the normal male and 1.35% for the untreated castrates. In the older group *D41* had a renal weight nearly double that of the untreated, castrated litter-mate *D40*. This very marked increase in renal weight seemed to be caused by tubular hypertrophy. The cells of the uriniferous tubules increased enormously in size; the cytoplasm appeared normal, and brush borders were readily visible in the proximal convoluted tubules, but the cells were so large that they protruded into the lumen, thus decreasing the internal diameter of the tubule. Debris plugged partly or completely almost all of the convoluted tubules, but the lumina of the loops of Henle and of the collecting ducts were relatively empty. Hypertrophy seemed to involve all parts of the kidney epithelium and include even the thin layer of cells lining the pelvis. Evidence that this is an hypertrophy and not merely a hyperplasia lies in the fact that the nuclei are not more numerous in cross sections of tubule, but are merely pushed farther apart by the increased amounts of cytoplasm. Figure 1 shows the degree of hypertrophy of the tubule cells of the testosterone treated castrates as compared with those of an untreated control castrate.

A marked degree of hydronephrosis existed in all the animals treated with testosterone propionate, but complete bladder obstruction was demonstrated in only one case (*D41*). This animal had shown evidences of uremia (diaphoresis, stupor and scattered areas of atrophy of the skin) for two or three days before it was killed. The extremely high kidney weight:body weight ratio which was obtained may have been caused in part by the extensive emaciation accompanying this condition. Postmortem examination revealed a urinary bladder filled with urine and distended to the level of the xiphoid process. The degree of hydronephrosis resulting, however, was not much greater than that found in the animals which did not manifest complete obstruction. Figure 2 shows the cross section of a kidney of a castrate treated with testosterone propionate but showing no overt symptoms of urinary obstruction compared with a similar section through the kidney of an untreated castrate.

The third effect of the treatment of castrate mice with testosterone propionate is the increase in the percentage of cuboidal cell capsules. As already noted, the percentage of high cell capsules in a normal male mouse of 10 to 11 weeks of age is 80% as compared with an average of 27% in castrates. The injection of a comparable group of 5 castrates with testosterone propionate resulted in a rise in the percentage of cuboidal cell capsules to an average for the group of 67%. In the older hybrid strain similar results were obtained. The kidney of a normal male of 11 weeks and 2 days contained 84% cuboidal cell capsules, a 13-week-old male castrated for 8 weeks showed only 23%, and in the kidney of a litter-mate castrated at the same time but injected for 3 weeks with testosterone propionate the value was 76%. Thus castration and the resultant absence of large amounts of the male sex hormones tends to retard the development of cuboidal cell capsules so that the percentage remains similar

to that of the female, while treatment with testosterone propionate stimulates their normal development to a level resembling that of the normal male.

The average total number of capsules counted in 12 sections of the untreated castrates' kidneys was in general considerably higher than the total number counted in an equal number of sections of the treated animals. Thus in the full-grown, treated animals the number of Bowman's capsules probably does not increase following hormone administration; the capsules are merely pushed farther apart by the tubular hypertrophy so that fewer are included in a single section.

C. *The Relationship between the structure of the kidney and the development of the adrenal gland.* As predicted by Howard (10) and Gersh and Grollman (11), the dark-staining androgenic zone of the adrenal of the mice castrated at 3 weeks of age persisted more or less unchanged, and 7½ weeks following castration the adrenals as well as the kidneys resembled those of the female control. On the other hand, in castrates of the same age which had been receiving testosterone propionate injections for 3 weeks previous to death, the androgenic zone had degenerated and was replaced by a connective tissue band separating the cortex and the medulla, as was the condition in the normal male control (table 1). Thus the changes in the kidney cortex caused by castration and subsequent hormone treatment are paralleled by the logical and predicted changes in the adrenal gland.

DISCUSSION

The weight increases obtained in the testosterone treated castrates' kidneys in the preceding study are in accord with the results of Selye (7) and Pfeiffer, Emmel, and Gardner (8). The latter have demonstrated, by comparing fresh and dry renal weights, that the increase in the size of the cells was a true hypertrophy and not merely a storage of fluid without a corresponding increase in the solids.

Since uniform results were obtained in both experimental groups, the time of castration seems to make little difference. In both groups the kidneys of the castrates tended to resume the immature form, and comparable responses to hormone injections were obtained: the castrates' kidneys tending to return to nearly the normal male condition. Testosterone propionate in the doses used in this experiment, however, failed to completely reproduce the male condition, since in none of the animals did the percentage of cuboidal cell capsules rise quite as high as in the normal male. There is little possibility that this failure is owing to underdosage, since the lowest value for the percentage of cuboidal cell capsules was found in the pair of animals which received the greatest daily dose. It is more probable that massive doses have a depressant effect much as they tend to depress the general body growth (13). It is possible that a greater percentage of cuboidal cell capsules might have been obtained by using smaller dosages of testosterone propionate over a longer period of time, and more closely approximating the normal physiological condition for mice of that age.

Complete obstruction of the urethra accompanied by enlargement of accessory reproductive organs which compressed the contents of the pelvis was demonstrated in only one animal (*D41*). The degree of hydronephrosis, however, did not appear noticeably greater than in some of the other animals where no such obstruction could be demonstrated and where the bladder was not enlarged, Burrows (12) noted a similar condition of hydronephrosis in male mice treated with estrogenic compounds, even when there was no evidence of enlargement of the bladder or obstruction of the urethra. He suggests the failure of the neuro-musculature apparatus to function normally as the cause of the hydronephrosis.

It is to be noted that castration produces in the kidney of the male a return to the immature male or to the female condition, but does not result in the complete absence

of cuboidal cell capsules. The explanation of this finding is not clear at this time. The extremely close chemical relationship between estradiol, progesterone, estrone, testosterone, androsterone, some of the adrenal cortex hormones and cholesterol, together with the fact that renal hypertrophy has been obtained with some of the other sterols (8) suggests that other hormones may be present which are capable of producing atony to a lesser degree.

It is possible that the tissues of mice are particularly sensitive to endocrines and under their influence undergo extensive histological and cytological changes. Alterations just demonstrated in the structure of Bowman's capsule of treated and untreated castrates, as well as the accompanying changes in the adrenal are evidences of such sensitivity. It has been known for a number of years that in the human being pregnancy causes a marked increase in the size of the fibrous and muscular coats of the juxtavesical portion of the ureters (14). In young pregnant mice premature degeneration of the androgenic zone of the adrenal accompanies the alteration in the amounts and types of endocrines secreted (2). Data are being collected at the present time to determine whether the method of differential count of the two types of capsules will reveal a response of the kidney cortex in mice to changed endocrine relationships that exist in pregnancy and are correlated with degeneration of the androgenic zone.

SUMMARY

Since the differential count of the two types of Bowman's capsules has proven to be a reliable index of age and sex variations in normal mice, a study was made using this method to estimate the effects on the kidney of castration and castration followed by the administration of testosterone propionate.

Castration affects the mouse kidney by causing a slight decrease in the kidney weight:body weight ratio and a fall in the percentage of cuboidal cell capsules to approximately the female level for animals of the same age.

The administration of testosterone propionate to castrated mice results in a marked hypertrophy of all parts of the kidney, hydronephrosis, and a rise in the percentage of cuboidal cell capsules to nearly the normal value for the male of the same age.

The decrease in the percentage of cuboidal cell capsules in the mouse kidney following castration is accompanied by a regeneration of the x-zone or androgenic zone of the adrenal. Treatment of the castrates with testosterone propionate causes the percentage of cuboidal cell capsules of the kidney to rise and at the same time the androgenic zone degenerates again, thus indicating that the induced changes in the kidney are closely correlated with changes in the androgenic zone of the adrenal.

The author wishes to express her appreciation to Dr. E. Granville Crabtree, for suggesting this problem and to Professor Myra M. Sampson for her generous interest and assistance in the direction of the research.

REFERENCES

1. Crabtree: C. E.: *Anat. Rec.* 79: 395. 1941.
2. Deanesly, R.: *Proc. Roy. Soc. B.* 103: 523. 1928.
3. Noble, G., and B. Greenburg: *Proc. Soc. Exper. Biol. & Med.* 44: 460. 1940.
4. Korenchevsky, V., M. Dennison and A. Kohn-Speyer: *Biochem. J.* 27: 557. 1933.
5. Korenchevsky, V., and M. Dennison: *Biochem. J.* 28: 1486. 1934.
6. Korenchevsky, V., M. Dennison and S. Simpson: *Biochem. J.* 29: 2534. 1935.
7. Selye, H.: *J. Urol.* 42: 637. 1939.
8. Pfeiffer, C., V. Emmel and W. Gardner: *Yale J. Biol. & Med.* 12: 493. 1940.
9. Ludden, J., E. Krueger, and I. Wright: *J. Clin. Invest.* 19: 780. 1940.
10. Howard, E.: *Am. J. Anat.* 65: 105. 1939.
11. Gersh, I., and A. Grollman: *Anat. Rec.* 75: 131. 1939.
12. Burrows, H.: *Proc. Roy. Soc. Med.* 29: 404. Part I, 1935–1936.
13. Rubinstein, H., and M. Solomon: *Proc. Soc. Exper. Biol. & Med.* 44: 442. 1940.
14. Hofbauer, H.: *Bull. Johns Hopkins Hosp.* 42: 118. 1928.

INFLUENCE OF PREGNANCY ON THE RENAL CORTEX OF MICE AS INDICATED BY THE STRUCTURE OF BOWMAN'S CAPSULE[1]

CHARLOTTE E. CRABTREE
From the Department of Zoology, Smith College
NORTHAMPTON, MASSACHUSETTS

As MENTIONED in a previous paper (1) the renal cortex of normal mice (of both sexes (2, 3), and normal and gonadectomized rats (4, 5) responds to the administration of several of the steroid sex hormones by an increase in absolute and relative weight. In certain mammals, including mice but not rats, the parietal lamina of some of the Bowman's capsules is normally composed of cuboidal cells instead of the squamous epithelium generally described in textbooks of mammalian histology. Selye (2) and Pfeiffer, Emmel and Gardner (3) noted the presence of large numbers of these cuboidal cell capsules in addition to renal enlargement in their treated animals. Crabtree (6) has demonstrated that in both sexes the percentage of cuboidal cell capsules in the normal mouse kidney increases with age, but at all times after puberty the renal cortex of the normal male contains a relatively greater number of cuboidal cell capsules than the cortex of a normal female of the same age.

Further study indicates that in the male mouse the relative numbers of the two types of capsules are influenced by the amount of androgen present. In place of the typical, marked, postpubertal rise in the relative number of cuboidal cell capsules of the normal male, in 10-week-old male castrates the percentage of these in the renal cortex showed little increase above that exhibited at the time of castration (3 weeks of age), and a value slightly less than that of the normal female of the same age as the castrates when killed. The injection of similar castrates with varying doses of testosterone propionate resulted in a tubular hypertrophy and hydronephrosis similar to that described above (2, 3). In addition the percentage of cuboidal cell capsules of these treated castrates showed a marked and significant increase over the values manifested by the untreated castrates, and was only slightly lower than that of the normal male of the same age (1).

A correlation seems to exist between the number of cuboidal cell capsules in the renal cortex and the presence and time of degeneration of the androgenic or x-zone of the adrenal gland first described by Howard-Miller (7) and Deanesly (8). In both the normal and the experimental animals the degeneration of the androgenic zone is coincidental with an abrupt rise in the relative number of cuboidal cell capsules. Further details of this relationship may be found in the discussion of this paper.

Thus the relative number of cuboidal cell capsules of the mouse kidney appears to alter under varying hormonal conditions, and can therefore be used as an index of hormonal effects on the renal cortex. Furthermore, an increase in the percentage of cuboidal cell capsules seems to be concommitant with the degeneration of the androgenic zone of the adrenal. It has been known for a number of years that pregnancy in young female mice is accompanied by a premature degeneration of the androgenic zone (9, 10, 11). Two of the hormones recovered from the urine during pregnancy

Received for publication May 21, 1941.
[1] Contributions from the Department of Zoology, Smith College, No. 196.

are progesterone, secreted in large amounts during the first part of gestation by the corpus luteum, and emmenin, an ester of estriol (theelin) secreted by the placenta during the latter part of pregnancy. Both of these hormones are derived from cholesterol and closely related chemically to testosterone (12). In view of these facts the following study was made to determine whether the influence on the renal cortex of these or other hormones occurring in normal pregnancy might be demonstrated in mice by the aforementioned method of differential counts of the two types of Bowman's capsules.

MATERIAL AND METHODS

The mice used in this experiment were hybrid albinos from the Smith College animal colony, weaned at about 4 weeks of age and maintained on a stock diet of Purina puppy biscuit. Estrus periods were determined by the vaginal smear technic (13), the vagina was washed out daily with a few drops of 0.8% NaCl in a dropping pipette; the washings were transferred to a clean slide and examined unstained with a compound microscope before the material dried. Females were placed with males only when the smear contained exclusively cornified epithelium, and they were removed as soon as a vaginal plug was obtained or leucocytes reappeared. Those animals killed at various intervals in the puerperium were allowed to nurse their young, although *mouse D65* failed to do so and the young all died at some time during the first week after birth.

Twenty-three females from 4 different litters were used in the series. Since the number of cuboidal cell capsules is known to increase with age, an attempt was made to obtain a series of animals to be killed at 11 to 12 weeks of age (just before the androgenic zone begins to disappear), and at different stages of pregnancy and of the puerperium. (See table 1 for the distribution of stages studied.) From each litter at least one non-pregnant female of approximately the same age as the experimental animals was killed as a control. Two animals (*D76* and *D46*) were somewhat younger than the rest of the group, but their records are included for whatever value they may have. In spite of age differences which would tend to lower the percentage of cuboidal cell capsules found, these figures from the above 2 animals agree closely with the results obtained from the older animals of the series.

Histological methods for the preparation and the examination of the kidneys have been described in detail elsewhere (6). In most of the animals the adrenals were also preserved and sectioned with the kidneys so that the condition of the androgenic zone of the individual animals could be correlated with the percentage of cuboidal cell capsules found.

RESULTS

The results of this study are summarized in table 1 and in the accompanying graph. The pregnancy period (21 days in the mouse) has been divided into trimesters of 7 days each, corresponding to the 3-month trimesters of human pregnancy. Records are also included of the conditions found in the first week of the puerperium (a period corresponding to the first 3 months postpartum in the human being).

The relative number of cuboidal cell capsules averaged 38% for the group of normal, non-pregnant control animals of the series. In animals killed during the first part of pregnancy this value increased rapidly and reached a peak of 69% at the end of the first trimester and the beginning of the second. A gradual decrease was observed during the remainder of the gestation period until by the eighteenth day the average percentage of cuboidal cell capsules was again equal to the average for the control group. Animals of the postpartum group showed no significant variation from the normal condition for mice of that age and sex.

The androgenic zone was present and well-developed in all the non-pregnant controls, and in the earliest stages of pregnancy. Degeneration occurred quite suddenly between the ninth and eleventh days of the gestation period. With the single exception of a few glandular cells observed among the connective tissue fibers in the adrenal of the 16-day pregnant animals, the remainder of the series had adrenals in which the androgenic zone had been completely replaced by a well-differentiated band of connective tissue which separated the cortex of the gland from the medulla.

TABLE 1. THE RESULTS OF STUDY OF THE EFFECT OF PREGNANCY AND THE PUERPERIUM ON THE STRUCTURE OF BOWMAN'S CAPSULES IN THE RENAL CORTEX OF MICE

Series No.	Litter No.	Age in weeks-days	Condition	No. high cell cap.	Total no. cap.	% High cell cap.	Condition of the X-zone
D56	1	11-0	Non-pregnant controls	583	1711	34	Clearly differentiated
D64	1	11-4		330	1234	27	Clearly differentiated
D51	2	12-0		491	1448	34	Present but narrow
D49	2	12-0		737	1645	55	—
D79	3	11-3		581	1390	42	Few strands of fibers
D69	4	12-1		520	1387	38	Few strands of fibers
			days pregnant				
D77	3	10-4	2	790	1638	48	Few strands of fibers
D46	2	9-5	6	769	1116	69	No fibers present
D61	1	11-4	9	893	1394	64	Degeneration proceeding
D57	1	11-4	10	843	1351	62	Only a few gland cells
D59	1	11-4	11	915	1327	69	Completely degenerated
D50	2	11-6	12	637	1150	55	—
D47	2	11-6	13	733	1157	63	Completely degenerated
D48	2	11-6	15	761	1231	62	Completely degenerated
D58	1	11-3	16	577	1053	55	Few gland cells
D55	1	11-1	18	581	1494	39	Completely degenerated
			days postpartum				
D72	4	12-2	0.5	502	1395	36	—
D76	3	8-6	1	590	1461	40	Completely degenerated
D68	4	11-6	1	482	1361	35	Completely degenerated
D71	4	12-2	1.5	528	1528	35	Completely degenerated
D66	4	12-1	3	646	1445	45	Completely degenerated
D67	4	12-0	4	436	1595	27	Completely degenerated
D65	4	12-2	7	756	1759	43	Completely degenerated

DISCUSSION

It is a well-known fact that human individuals may vary in their response to pregnancy, i.e. the degree of muscular atony, the extent of pigmentation, and the general antepartum softening of certain connective tissues of the body. The small fluctuations in the curve of results obtained in this study might be explained on this basis.

The foregoing experiment verifies earlier observations (9, 10, 11) that pregnancy causes a premature degeneration of the androgenic zone of the adrenal. The results show that this degeneration occurs at the beginning of the second trimester of pregnancy, and that at this time the percentage of cuboidal cell capsules is at its highest point. This correlation between degeneration of the androgenic zone and the high percentage of cuboidal cell capsules is in accord with C. E. Crabtree's previous results (1, 6).

The relationship between these two events is still not clear. The fact that the rise in the relative number of cuboidal cell capsules occurs almost exclusively in the first half of pregnancy would seem to indicate that the corpus luteum hormone, progesterone, might be responsible for the changes. Howard and Gengradon (11), however, have demonstrated that progesterone and estrogen in pure form do not cause appreciable, pregnancy-like degeneration of the androgenic zone, although testosterone and androsterone do. In view of Hill's (14) experiments demonstrating that ovaries grafted into the ears of castrate male mice are capable of secreting enough male hormone to restore the seminal vesicles and prostate to a normal condition, Howard and Gen-

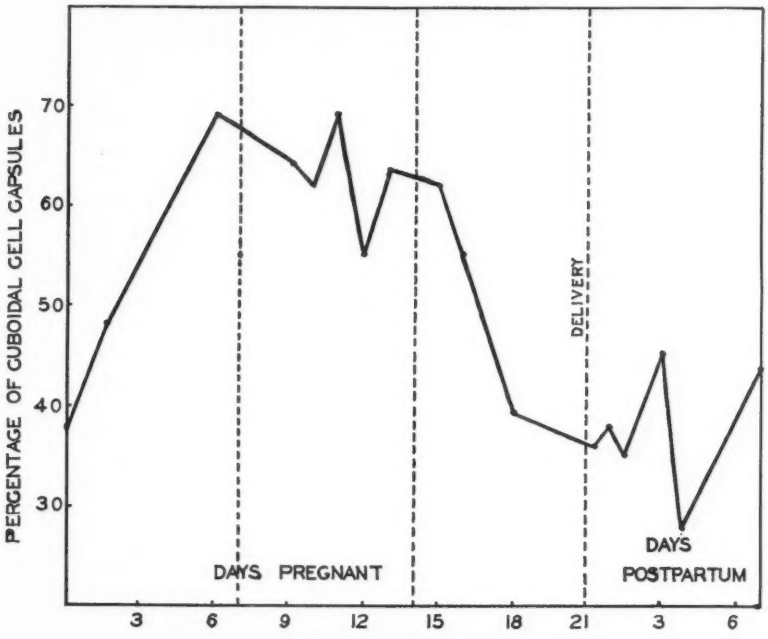


Fig. 1. Effect of pregnancy on the Bowman's capsule in mice

gradon (11) postulate a general increase in the "maleness" of the female mouse during pregnancy, possibly owing to the increased production of androgens by the ovary under this condition, or possibly correlated with the relatively large amount of developing male tissue of the embryos. The increase in the percentage of cuboidal cell capsules observed in this experiment is in accordance with this hypothesis; the fact that the greatest change occurs during the first half of gestation might be interpreted as supporting the first rather than the second suggested explanation.

The influence of pregnancy on the efferent channels of the urinary tract is well known. Characteristic dilatation of the anterior third of the ureters and of the kidney pelvis is accompanied by an increase in the total length of the ureters, their lateral displacement, and frequently by kinking. In women, microscopic variations include hypertrophic and hyperplastic changes of both the smooth muscles and the connective tissue particularly in the juxtavesical and intravesical portions of the ureters and of the bladder trigone (15).

Because of anatomical differences, pregnancy in the quadripedal mammals cannot be compared directly with pregnancy of the bipedal human being. In pregnant women the compression of the ureters between the brim of the unyielding bony pelvis and the enlarged, pear-shaped uterus is undoubtedly a factor in producing the dilatations. In lower mammals where the embryos are carried in the horns of the Y-shaped uterus and the weight is supported by mesenteries and the lax abdominal wall rather than by the pelvis and muscles of the perineum, mechanical compression of the ureters does not occur, and dilatations are not normally found (15, 16, 17). Mengert (16) found no very significant and constant histological changes in the ureters of a series of pregnant quadripedal mammals.

Physiological relationships, however, are more clearcut. Baird (18), Hortolomei (19), and others called attention to the fact that in the human being dilatations of pregnancy were frequently more extensive than those caused by cysts or tumors of similar size and location. In the last twenty years increasing importance has been attributed to the role of pregnancy hormones in producing a physiological atony of the smooth muscles of the walls of the ureters, and thus allowing the dilatation to proceed to greater than normal limits. The action of the hormones, folliculin, progesterone and the gonadotropic hormone of the pituitary, on isolated pig ureter is to diminish peristalsis (20). Similar results were obtained by Villaret (21) on sections of dog ureter. Both Bompiani (22) and Contiades (23) report that the urine of women obtained during the middle months of pregnancy when the maximum changes in the urinary tract occur and when the most estrin is present in the blood (24), causes a corresponding inhibition of ureteral peristalsis.

Further evidence of the atony induced during pregnancy in both the human being (25) and in lower mammals (26) may be obtained by the pyelographic study of pregnant and nonpregnant individuals. E. G. Crabtree, Abramson, and Robins (17) attempted to reproduce in rabbits the pressure factor present in the human being by applying mechanical pressure to the abdominal contents and the ureters just above the pubic symphysis, and found that in pregnant animals a greater dilatation and more complete filling of the ureters occurred than in the same animals in a non-pregnant condition. This indicates that muscular atony also exists in the lower animals, although the lack of mechanical compression of the ureters fails to translate this atony into a passive dilatation.

The curve of change in the percentage of cuboidal cell capsules obtained in this study is similar to curves obtained by other workers studying different phases of pregnancy. In nearly every case the greatest change has been demonstrated to occur during the middle trimester of the gestation period, and a tendency is noted to return toward the normal condition just before delivery. In women dilatations and histological changes in the efferent channels are known to be greatest from the third to the eighth month of pregnancy; Traut, McLane and Kuder's curve of ureteral activity during pregnancy follows this same pattern (27), as does Baird's study of the appearance time of intravenous dye observed by cystoscopic methods (18). Among the lower mammals the curve maintains the same general shape, although it may be shifted in position to a slightly earlier or a slightly later phase of the pregnancy period. The greatest increase in cuboidal cell capsules in the mouse kidney (as seen in fig. 1) falls somewhat early in the gestation period, and the return to normal is complete before delivery. In the monkey, dilatations of the efferent channels are most likely to occur slightly past the mid-pregnancy stage (28), and Rossi (26) finds the greatest changes in rabbits to occur at about the twenty-third day of a 30-day pregnancy period.

The effect of pregnancy upon the renal cortex is more obscure. The literature shows no reference to histological or structural changes in either the human being or

in lower mammals. In women, however, studies of the after effects of infections of the renal cortex during pregnancy show that this tissue is apparently particularly vulnerable during this period; in many cases a far greater functional damage has resulted than one would be warranted in expecting from the apparent severity of the initial infection (29, 30).

Thus the renal cortex, in addition to the efferent channels, seems to be affected by pregnancy, although the exact nature of this change is still unknown. The foregoing observations establish the existence, in mice, of definite histological changes occurring as a response to normal pregnancy. It is logical to postulate that in the human being a similar response of the renal cortex to hormones of pregnancy occurs, although, as has been shown to be the case in pregnancy changes of the efferent channels, it is not necessarily manifested in the same way.

SUMMARY

In a series of young female mice of the same age but in different stages of pregnancy or the puerperium, an increase in the percentage of cuboidal cell capsules of the renal cortex was found to occur during the first half of pregnancy. The maximum value was reached at the end of the first trimester and the beginning of the second. It then decreased to normal by the eighteenth day of a 21-day gestation period.

There was no significant alteration observed in the percentage of cuboidal cell capsules during the puerperium.

The androgenic zone of the adrenal gland degenerated abruptly at about the tenth day of pregnancy when the percentage of cuboidal cell capsules was at the maximum.

The similarity between pregnancy changes in human beings and in lower mammals has been demonstrated by a number of investigators. An effect of pregnancy on the renal cortex of the human analogous to the change just demonstrated in mice might be expected to occur, but this change is probably not manifested in the same way.

REFERENCES

1. Crabtree, C. E.: *Endocrinology* 29: 197. 1941.
2. Selye, H.: *J. Urol.* 42: 637. 1939.
3. Pfeiffer, C. A., V. M. Emmel and W. U. Gardner: *Yale J. Biol. & Med.* 12: 493. 1940.
4. Ludden, J., E. Krueger and I. Wright: *J. Clin. Invest.* 19: 780. 1940.
5. Korenchevsky, V., and M. A. Ross: *Brit. Med. J.* 1: 645. 1940.
6. Crabtree, C. E.: *Anat. Rec.* 79: 395. 1941.
7. Howard-Miller, E.: *Am. J. Anat.* 40: 251. 1927.
8. Deanesly, R.: *Proc. Roy. Soc. B.* 103: 523. 1928.
9. Gersh, I., and A. Grollman: *Anat. Rec.* 75: 131. 1939.
10. Howard, E.: *Am. J. Anat.* 65: 105. 1939.
11. Howard, E., and S. Gengradon: *Endocrinology* 26: 1049. 1940.
12. Cameron, A. T.: Recent Advances in Endocrinology, Blakiston Co., Philadelphia, 1940.
13. Stockard, C. R., and G. N. Papanicolaou: *Am. J. Anat.* 22: 225. 1917.
14. Hill, R. T.: *Endocrinology* 21: 495. 1937.
15. Payne, F. L., and J. P. Hodes: *Am. J. Obst. & Gynec.* 37: 1024. 1938.
16. Mengert, W. F.: *Am. J. Obst. & Gynec.* 27: 544. 1934.
17. Crabtree, E. G., D. Abramson and S. H. Robins: *Surg. Gynec. & Obst.* 71: 60. 1940.
18. Baird, D.: *J. Obst. & Gynec. Brit. Emp.* 43: 1. 1936.
19. Hortolomei, N., Th. Burghele and M. Streja: *J. d'Ur l.* 43: 399. 1937.
20. Grossu-Strenja, E., and I. D. Georgesco: *Compt. Rend. Soc. de Biol.* 119: 628. 1935.
21. Villaret, M.: *Compt. Rend. Soc. de Biol.* 117: 1165. 1934.
22. Bompiani, R.: *Ann. di Ostet. e Ginec.* 55: 1755. 1933.
23. Contiades, V. J.: *J. Phisiol. et Path. Gen.* 33: 913. 1935.
24. Reinburger, J. R., and R. E. Anderson: *Nebraska M. J.* 22: 328. 1937.
25. Crabtree, E. G., G. E. Prather and E. L. Prien: *J. d' Urol.* 38: 605. 1937.
26. Rossi, V.: *Arch. Ital. di Urol.* 13: 232. 1936.
27. Traut, H. F., C. M. McLane and A. Kuder: *Surg. Gynec. & Obst.* 64: 51. 1937.
28. Van Wagenen, G., and R. H. Jenkins: *J. Urol.* 42: 1010. 1939.
29. Crabtree, E. G., and D. E. Reid: *Am. J. Obst. & Gynec.* 40: 17. 1940.
30. Crabtree, E. G.: *J. Urol.* 44: 125. 1940.

PROGESTERONE-LIKE EFFECT OF ASCORBIC ACID (VITAMIN C) ON THE ENDOMETRIUM

S. LEON ISRAEL AND DAVID R. MERANZE

From the Department of Gynecology and the Laboratories, Mount Sinai Hospital

PHILADELPHIA, PENNSYLVANIA

EVIDENCE has accumulated in the past few years to indicate that the corpus luteum contains other active substances besides progesterone and an estrogen (1, 2). One of these alleged additional principles is ascorbic acid (vitamin C).

It has been shown by several investigators that the corpus luteum has a high content of ascorbic acid, the quantity of which parallels the degree of luteal development (3–8). The remarkable correlation between the concentration of ascorbic acid and the growth of the corpus luteum is best exemplified in the difference between the non-pregnant and the pregnant animal. The corpus luteum of pregnancy contains twice as much ascorbic acid as that found in the mature corpus luteum of non-pregnancy (6, 8). These findings suggested to some investigators the possible existence of a causal relationship between the rate of growth of the corpus luteum and its content of ascorbic acid. Such a relationship was further supported by the observation that a diet deficient in vitamin C results in degeneration of corpora lutea and abortion in guinea pigs (9–10). Ley (8), hypothecating that ascorbic acid may stimulate a failing corpus luteum, successfully treated 10 women with habitual abortion by means of vitamin C.

In view of these suggestive, but by no means conclusive, data, it seems reasonable to believe, as suggested by Bourne (11), that vitamin C is related in some manner to the production of the corpus luteum hormone, progesterone. It appeared to the authors that some light might be thrown on this problem by an attempt to reproduce some of the physiologic responses of progesterone by means of ascorbic acid. This study of the comparative effects of ascorbic acid and of progesterone on the endometrium was undertaken for that reason.

METHODS

Experiments were carried out in intact infantile rabbits and in ovariectomized adult mice, rats and rabbits. The familiar technic employed in the bioassay of progesterone, namely, estrogen-priming followed by the administration of progesterone, was used. The control animals received either estrogen alone or estrogen and progesterone; whereas, the test animals received estrogen followed by ascorbic acid.

The adult animals were ovariectomized and permitted to recover during a period of 3 weeks. All of the animals, infantile and adult, were primed by daily subcutaneous injections of estrogen for 7 days. Half of each group, numbering from 16 to 20 animals, were then given daily subcutaneous injections of ascorbic acid for 5 days. The remaining half served as controls: one-quarter received no further injections (estrogen controls) and the remaining fourth was given daily subcutaneous injections of pro-

Received for publication May 5, 1941.

gesterone (progesterone controls). All animals were killed on the day following the last injection. The uterine horns were removed, weighed, and prepared for histologic study.

The estrogen employed in priming the animals was a standard brand of estradiol dipropionate, in sesame oil, with an assay value of 3,000 R.U. per mg. The mice were primed with 1 R.U. (0.00035 mg.) daily, the rats with 2 R.U. (0.0007 mg.), and the rabbits with 15 R.U. (0.005 mg.). The progesterone used was a standard brand of synthetic origin, in sesame oil. The quantity injected daily was 0.25 mg. in the mice, 0.5 mg. in the rats, and 2.0 mg. in the rabbits. The ascorbic acid was used in the form of the neutral, stable, water-soluble, nontoxic monoethanolamine salt[1] which is reported to be just as effective as the acid itself (12). The mice received 50 mg. daily, the rats 100 mg., and the rabbits 500 mg.

RESULTS

Infantile rabbits. On macroscopic examination, the uterine horns of the progesterone treated infantile rabbits were obviously larger and more hyperemic than the uteri of the other 2 groups. The average uterine weight of the estrogen control series was 2.2 gm., of the vitamin treated animals 3.2 gm., and of the progesterone controls 5.7 gm.

Definite histologic differences were noted in the uterine horns of the 3 series of infantile rabbits. Inspection of the mounted specimens with a hand lens disclosed the relative paucity of glands in the endometrium of the estrogen controls, the evident increase in the number of glands and their rudimentary branching in the ascorbic acid-treated series, and the lush lace-like pattern of the progesterone controls (fig. 1–3).

Microscopically, the glandular epithelium of the 3 groups of uteri exhibited sharp variations, the change in the vitamin treated rabbits being intermediate between the estrogen and the progesterone controls. The epithelium lining the glands in the endometrium of the estrogen control rabbits was cuboidal. The nuclei were relatively large, round and vesicular, possessing no regular position in the cells. The cytoplasm was homogeneous and agranular. The lumen surface of the glands was smooth and unbroken (fig. 4). The markedly altered endometrium of the progesterone treated group showed a highly functional type of glandular epithelium. The cells were of the tall columnar type, with granular cytoplasm. Their relatively small nuclei were oval, compact, and aligned just above the basement membrane. The lumen surface of the glands showed a wavy appearance (fig. 5). The endometrial pattern of the vitamin treated rabbits showed an approximation of the changes observed in the progesterone treated animals. The lining cells were columnar, the dense nuclei were basally situated and the lumen surface of the cells was frayed (fig. 6).

The only alteration of note in the stroma and the muscle layers of the uterine horns was the intense hyperemia, as evidenced by the dilated capillaries, in the progesterone treated animals—a phenomenon present to some extent in the uteri of the vitamin treated group.

Ovariectomized adult rabbits. The uterine horns of the estrogen control group of spayed adult rabbits appeared, on gross inspection, smaller and paler than the horns of the other 2 groups. This difference was more apparent in the comparison of the average uterine weights. The average uterine weight of the estrogen control group was 2.8 gm., that of the ascorbate treated animals 4.2 gm., and that of the progesterone

[1] Monoethanolamine salt of ascorbic acid, in the form of Cenolate, was supplied by the Abbott Laboratories, North Chicago, Illinois through the courtesy of Dr. J. F. Biehn. This preparation contains 76% ascorbic acid. It is dispensed in 2 cc. ampoules, each containing 100 mg. (2,000 U.S.P. units).

controls 4.7 gm. A similar division of the material was possible microscopically, the uterine horns of the ascorbic acid treated animals resembling those of the progesterone controls (fig. 7–9).

The endometrium of the progesterone treated adult rabbits did not, as in the infantile rabbits, exhibit the lace-like pattern which is the familiar end-point of progesterone assays. The glandular structure, which was markedly increased in compari-

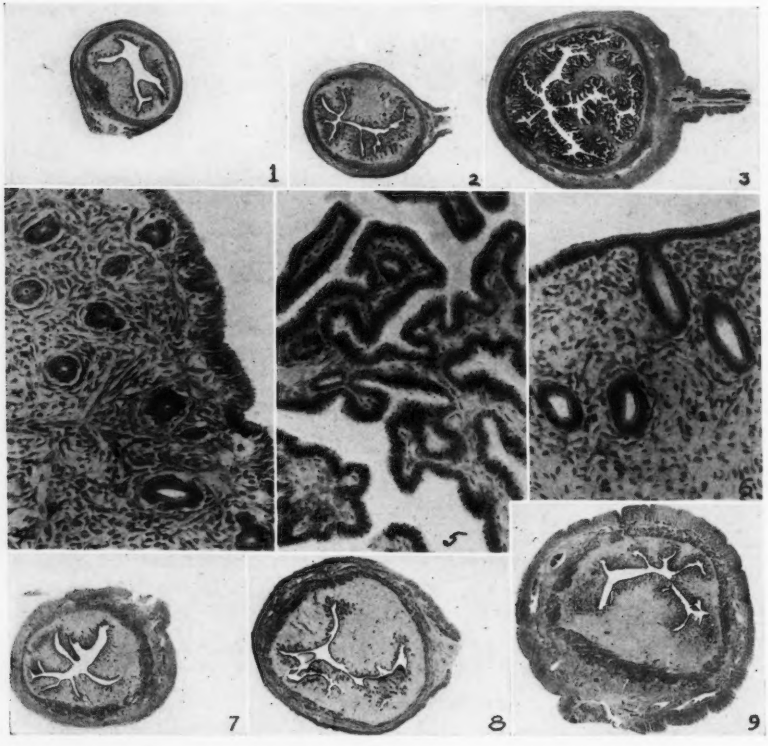


Fig. 1–3. Cross-sections of the uterine horns of the infantile rabbits. ×5.3. Fig. 1. Estrogen control. Fig. 2. Ascorbic acid treated. Fig. 3. Progesterone treated.

Fig. 4–6. Portion of the endometrium of the infantile rabbits. ×140. Fig. 4. Estrogen control. Fig. 5. Progesterone treated. Fig. 6. Ascorbic acid treated.

Fig. 7–9. Cross-sections of the uterine horns of the ovariectomized adult rabbits. ×5.3. Fig. 7. Estrogen control. Fig. 8. Ascorbic acid treated. Fig. 9. Progesterone treated.

son with that of the estrogen control group, did, however, show definite branching, a change to columnar epithelium, and the already noted nuclear modifications. The endometrium of the ascorbate treated rabbits showed similar alterations but to a lesser degree. The hyperemia in the endometrium of the 2 treated groups was not as marked as that noted in the endometrium of the treated infantile rabbits.

Ovariectomized adult rodents. Inasmuch as the findings in the mice and the rats were essentially the same, both groups are considered together. The uterine horns of the animals given progesterone were, upon gross examination, larger and more hyperemic. The average uterine weight of the progesterone treated animals also

exceeded that of the other 2 groups, the estrogen controls having the lowest average.

Histologically, the uteri of the progesterone treated rodents showed an altered glandular epithelium, the cells of which were pale, very tall, and possessed of small, dark, basally located nuclei. There was little, aside from slight changes in the glandular epithelium, to distinguish the endometrium of the estrogen controls from that of the ascorbate treated animals. There seemed, however, to be a tendency for the glandular epithelium of the latter animals to be taller and to show basally situated nuclei. This tendency was more apparent in the rats than in the mice. There were no stromal changes which could be used to identify the 2 treated groups from the estrogen control group. We were unable to find the increased mitosis in all the layers of the uterus, suggested by Hooker (13) as a specific morphologic response to progesterone in rodents.

DISCUSSION

The foregoing experimental observations demonstrate that the administration of empirically chosen quantities of ascorbic acid modifies the estrogen-primed endometrium in the direction ot the type induced by progesterone. It is possible that the use of larger quantities of vitamin C would have stimulated the endometrium to a greater degree.

In these studies, the rodent endometrium, as in the hands of other investigators (13), was least responsive to progesterone and was, therefore, a poorly chosen object for demonstration of the progesterone-like effects of ascorbic acid. The change to the secretory type of endometrium, following the exhibition of an ascorbic acid salt in an animal primed by estrogen, was most marked in the infantile rabbit—an animal known to respond readily to progesterone. The endometrial alterations were best studied microscopically under high power magnification, by means of which it was possible to discern the cellular mutations toward that of a secretory tissue. The transformation from cuboidal to columnar epithelium, the cytoplasmic swelling, and the nuclear alignment were especially noteworthy in the ascorbate treated infantile rabbits. The adult rabbits might possibly have proved better subjects if the ovariectomy and the administration of vitamin C had immediately followed mating, as in the Corner-Allen method of assaying progesterone.

The increase in the average uterine weight and the moderate stromal hyperemia in the ascorbate treated animals, while not equal in degree to the changes wrought with progesterone, were in consonance with the endometrial response.

The progesterone-like effect of ascorbic acid on the endometrium lends a measure of support to the belief that the finding of relatively large quantities of ascorbic acid in well-developed corpora lutea is biologically significant (3–8). It appears that the vitamin may be related physiologically to the corpus luteum hormone.

SUMMARY

The recent studies which demonstrated the high content of ascorbic acid in the corpus luteum and the alleged value of ascorbic acid in the treatment of habitual abortion led the authors to investigate the effects of ascorbic acid on the endometrium of infantile rabbits and of ovariectomized adult mice, rats and rabbits.

The animals were primed by means of daily injections of estrogen (estradiol dipropionate) for 7 days. The treated series were then given daily injections of the monoethanolamine salt of ascorbic acid for 5 days. The control animals received either no further injections (estrogen controls) or daily injections of progesterone for 5 days (progesterone controls). Comparison was then made of the average uterine weight, the gross appearance, and the microscopic characteristics of the uterine horns of each

group of animals. In the 3 series of animals, the progesterone controls developed the heaviest uterine horns and the estrogen controls the lightest, the vitamin treated animals being intermediate.

The endometrium of the vitamin treated animals approximated, particularly in the microscopic comparison, the secretory changes in the endometrium of the progesterone control group. This response was most marked in the infantile rabbits and least evident in the ovariectomized adult rodents.

REFERENCES

1. PHILLIPS, W. A.: *Am. J. Physiol.* 119: 623. 1937.
2. FREED, S. C., AND S. SOSKIN: *Proc. Soc. Exper. Biol. & Med.* 38: 391. 1938.
3. BESSEY, O. A., AND G. C. KING: *J. Biol. Chem.* 103: 687. 1933.
4. HUSZAK, S.: *Ztschr. f. physiol. Chem.* 219: 275. 1933.
5. GLICK, D., AND G. R. BISKIND: *J. Biol. Chem.* 110: 583. 1935.
6. BISKIND, G. R., AND D. GLICK: *J. Biol. Chem.* 113: 27. 1936.
7. PINCUS, G., AND J. BECKMAN: *Am. J. Physiol.* 119: 455. 1937.
8. LEY, L.: *Münch. med. Wchnschr.* 84: 1814. 1937.
9. KRAMER, M. M., M. T. HARMON AND A. K. BRILL: *Am. J. Physiol.* 106: 611. 1936.
10. VOGT, E.: *Münch. med. Wchnschr.* 81: 791. 1934.
11. BOURNE, G.: *Nature* 135: 148. 1935.
12. LOZNER, E. L., F. J. POHLE AND F. H. L. TAYLOR: *New Eng. J. Med.* 220: 987. 1939.
13. HOOKER, C. W.: *Proc. Soc. Exper. Biol. & Med.* 45: 270. 1940.

EFFECT OF ESTROGENIC SUBSTANCE ON EXPERIMENTALLY HYPERTHYROID MALE RATS[1]

T. C. SHERWOOD

From the Department of Medicine, University of Wisconsin

MADISON WISCONSIN

THE AUTHOR has investigated the thyroid-ovarian relationship in rats as measured by means of basal metabolic changes. Estrogenic substance, estrone, stilbestrol and estradiol were effective in the production of a decrease in the oxygen consumption of intact or ovariectomized animals made experimentally hyperthyroid as shown by Sherwood et al. (1–5).

The present investigation was undertaken for the purpose of producing a similar picture in the male rat and to determine whether sex need be considered in the use of estrogen therapy in hyperthyroidism.

EXPERIMENTAL

The details of feeding, temperature control, fasting period and time of day, which have been found essential for consistent results in the determination of basal metabolism (1), were observed in the present investigation.

All animals were 8 months old at the beginning of the experimental period. Twenty-seven male rats were used throughout the experiment. Ten of the animals were used as controls for the purpose of checking possible changes that might have occurred in technic and temperature variation. This procedure seemed advisable since males have responded less uniformly in the experience of the author.

Four intact animals were used in order to determine the extent of increase in basal metabolism following the administration of 1 gm. of desiccated thyroid per kg. body weight per day for a period of 3 days.

Seven animals were thyroidectomized following a series of normal basal metabolic determinations. Further metabolic readings were obtained until a consistent hypothyroid level had veen established. The rats were then fed 1 gm. of desiccated thyroid per kg. daily for a period of 3 days. Three of the above group were castrated as well as thyroidectomized.

A third group of 6 animals were castrated and thyroidectomized in order to obtain animals comparable to similarly treated females. These rats were injected with 1,000 I.U. of estrogenic substance (Amniotin) daily for a period of 9 days. One gram of desiccated thyroid per kg. was fed daily on the 4th, 5th and 6th days of Amniotin therapy. Daily basal metabolic determinations were obtained during the preliminary control period and continued until the hypothyroid level had again been established.

RESULTS

The intact animals returned to their original basal metabolic level within 8 days following the cessation of thyroid feeding. The thyroidectomized animals returned to the hypothyroid level in approximately the same time. However, the animals that had been castrated in addition to being thyroidectomized returned to the hypothyroid in 15 days following thyroid feeding.

[1] This research was supported by a grant from the Research Committee of the University of Wisconsin and the Wisconsin Alumni Research Foundation.

The average increase in basal metabolism of the intact animals was 60% while that of the thyroidectomized animals was 91%. The results were much more variable in the group of intact animals, the range being from 34 to 75% whereas the thyroidectomized animals showed a range from 83 to 100% (table 1).

The 6 animals which had been castrated and thyroidectomized showed an increase of 15 to 29% when estrogenic substance and desiccated thyroid were given simultaneously. The average rise in metabolism for this group was 19%.

DISCUSSION

In the present investigation, it was found that males responded less uniformly than females in the study of factors influencing the basal metabolism when similar procedures were used.

The intact males returned to the original basal metabolic level in approximately the same time required for females as shown by Sherwood and Bowers (1). The thyroidectomized-castrate males returned to the hypothyroid level in 15 days following the cessation of thyroid feeding in contrast to a period of 24 days for females as reported by Sherwood (3). It seemed advisable therefore to give the estrogenic substances and desiccated thyroid simultaneously in order to prevent a rise in metabolism as would be expected with thyroid substance alone. This procedure proved successful as shown in table 1. The full thyroid effect was not obtained when preceded and

TABLE 1. EFFECT OF ESTROGEN ON EXPERIMENTAL HYPERTHYROID MALE RATS

Animal	Weight	Decrease in B.M.R. due to thyroidectomy	Increase in B.M.R. due to thyroid therapy	Return to original B.M.R. level
	gm.	%	%	*days*
		Intact		
1	395		34	8
2	430		75	8
3	350		74	6
4	375		60	8
Average	387		60	7.5
		Thyroidectomized		
5	340	20	83	5
6	380	21	90	8
7	420	31	92	8
8	390	22	88	5
Average	382	23.5	88	6.5
		Thyroidectomized and Castrated		
9	350	33	92	16
10	375	27	100	17
11	360	20	95	15
Average	361	26.6	95.6	16
		Thyroidectomized and Castrated		
			Thyroid+estrogen	
12	335	28	15	2
13	360	22	29	7
14	320	26	20	6
15	340	21	18	5
16	410	24	16	4
17	390	22	15	8
Average	359	24	19	5.3

PROTOCOL 1. RAT 10. BASAL METABOLISM

Date	Weight	Oxygen	R.Q.	Calories/kg./hour	Remarks
	gm.	gm.			
11/ 6/40	320	0.85	0.73	4.52	Normal
11/13/40	318	0.85	0.74	4.44	Normal
Average				4.48	Normal
11/15/40					Thyroidectomy
2/11/41	395	0.85	0.76	3.58	
2/13/41	395	0.75	0.82	3.19	
2/15/41					Castrated
2/19/41	378	0.70	0.78	3.08	
2/21/41	369	0.75	0.73	3.33	
3/ 1/41	374	0.80	0.77	3.56	
3/ 4/41	380	0.70	0.83	3.11	
Average	375			3.27 (−27%)	
3/10/41					1 gm. thyroid/kg.
3/11/41					1 gm. thyroid/kg.
3/12/41	371	1.32	0.85	6.54	1 gm. thyroid/kg.
3/14/41	354	1.40	0.75	6.57 (+100%)	
3/16/41	349	1.15	0.76	5.48	
3/18/41	353	0.90	0.77	4.24	
3/22/41	351	0.85	0.77	4.03	
3/28/41	350	0.90	0.73	4.23	
3/29/41	347	0.80	0.73	3.49 (hypothyroid)	
4/ 3/41	347	0.75	0.78	3.60	
4/15/41	356	0.72	0.70	3.30	
4/17/41	353	0.75	0.70	3.47	

followed by Amniotin injections. This demonstrates that estrogenic substances can affect the basal metabolism of the male rat.

The author believes that the present investigation offers further proof that estrogens may have some practical application in the treatment of certain types of thyrotoxicosis. It is also believed that the results indicate a direct hormone antagonism in the tissues and that it is not due to an estrogenic action upon the thyroid, either directly or through the thyrotropic hormone.

SUMMARY

The present investigation indicates that animals of both sexes may be expected to respond to treatment of hyperthyroidism with estrogenic substances. Males are apparently affected in the same manner, when considering the thyroid-gonad relationship, as found in the female by means of basal metabolic changes.

The author wishes to express thanks to Dr. E. L. Sevringhaus for his generous suggestions and criticism during the period of investigation, and to Dr. J. A. Morrell of E. R. Squibb & Sons for the supply of Amniotin.

REFERENCES

1. SHERWOOD, T. C., AND L. M. BOWERS: *Am. J. Physiol.* 115: 645. 1936.
2. SHERWOOD, T. C., T. M. WILSON AND H. BONETA: *Am. J. Physiol.* 120: 671. 1937.
3. SHERWOOD, T. C.: *Am. J. Physiol.* 124: 114. 1938.
4. SHERWOOD, T. C.: *Endocrinology* 26: 693. 1940.
5. SHERWOOD, T. C.: *Endocrinology* 27: 925. 1940.

RELATION OF THE CHANGES IN TESTICULAR STRUCTURE INDUCED IN THE RABBIT BY ESTROGENIC SUBSTANCE TO RESISTANCE AGAINST SYPHILIS

C. N. FRAZIER, C. K. HU, AND W. C. MA

From the Division of Dermatology and Syphilology, Department of Medicine, and the Department of Anatomy, Peiping Union Medical College

PEIPING, CHINA

IN SEEKING the nature of the factors underlying the sexual differences in immunity to syphilis, we undertook an experimental study of the disease in male rabbits feminized by prolonged administration of an estrogenic substance (1, 2). This substance, prepared from a butyl alcoholic extract of human pregnancy urine, was capable of inducing estrous effects in ovariectomized rats. When given to adult male rabbits, there resulted a series of anatomical changes, including partial metamorphosis of the external genitalia, and the development of certain female secondary sex characters (1, 3).

When the testis was the site of inoculation, the feminized rabbits responded with a milder form of syphilis than the sexually intact male rabbits. This was true of both the primary testicular and the generalized metastatic phenomena of the disease.

In rabbits treated for a period of approximately 100 days, the testes showed definite signs of atrophy. Inoculated with syphilitic virus, they failed at times to develop orchitis, although it could be demonstrated that under such circumstances the animal harbored viable *Treponema pallidum*. Furthermore, in only one instance did the estrinized animals show a metastatic lesion of the uninoculated testis. It was apparent, also, that the longer the animals were subjected to estrogenic treatment, the less severe was the disease likely to become.

Judged by the refractory state of the testis in the estrinized animals, it seemed not unreasonable to assume that some function, or property, of the testicular tissues normally favors the growth and multiplication of *Treponema pallidum* when it is deposited in the testis either by direct inoculation or indirectly through the blood stream, and that this property is destroyed, or at least altered in its power to enhance the infection, as a result of estrogenic action. Inasmuch as the severity of the generalized manifestations of syphilis was also significantly modified by estrogenic treatment (2), the question naturally arose as to the influence the alterations of testicular structure had on the general resistance of the animal to infection. The importance of this question is emphasized by the fact that the reaction of the testis to syphilis in the rabbit apparently determines to a large extent the severity of the subsequent course of the disease; minor testicular lesions, as a rule, being followed by a severer form of generalized syphilis than when the primary orchitis is of greater magnitude or longer duration.

With the first of these questions in mind, we examined histologically the testes of the animals employed in the experiments previously described (1, 2). This we did to ascertain the effects both of estrogenic action and syphilitic infection. It was hoped that from the conjoined study of the two processes, infection and hormonal action, some knowledge might be gained of the identity of the cellular elements in the testis

which favor the pathogenicity of *Treponema pallidum* for this species. This paper is concerned with the results of the study.

MATERIAL AND METHODS

A detailed description of the experiments need not be entered into as they have already been adequately presented in the papers previously mentioned. It is sufficient to state that the testicular tissues examined came from 8 normal and 10 estrinized rabbits. The estrinized rabbits had been treated daily, 6 days a week, with subcutaneous injections of from 30 to 60 R.U. of estrogenic substance in olive oil. This was equivalent to approximately 100 to 200 I.U. of estrone daily. The period of treatment ranged from 105 to 331 days. The method of preparation of the estrogenic substance and its biological standardization have been described elsewhere (1). The normal rabbits had received, over the same period of time, injections of olive oil equivalent to those given together with the estrogens. Inoculation with *Treponema pallidum* was made into the right testis of each rabbit after 75 days of estrogenic therapy.

All of the untreated animals in the control group developed a primary orchitis at the site of inoculation, and 5 out of the 8 also developed a metastatic orchitis. Among the estrinized rabbits, however, only 4 of the 10 animals came down with a primary orchitis and but one developed an orchitis of the uninoculated testis. It was possible, therefore, to select from animals in each group, testes with and without demonstrable syphilitic lesions. This afforded an opportunity to distinguish between the effects of estrinization and infection, and provided a method for the identification of the changes in the cellular fabric of the testis induced by the one or the other process.

Fixation and staining. The testes were removed, weighed, fixed in Zenker-formol solution and imbedded in paraffin. Transverse sections were cut from 3 different levels, and stained with hematoxylin and eosin. In addition, selected specimens were treated with Mallory's connective tissue stain, Heidenhain's iron hematoxylin and methyl-green-pyronin. On the whole, the last two methods of staining did not yield any information not obtainable from the use of hematoxylin and eosin, and of Mallory's technic.

Measurement of areas of testis. Six sections from each testis, selected from each of 3 different levels, were used for measuring the areas occupied by the seminiferous tubules and the interstitial tissue respectively. Camera lucida drawings were made of representative areas from each transverse section. With a planimeter, the tracings were measured with respect to the total testicular area and to the area of the seminiferous tubules. The difference between the two was taken as the area of interstitial tissue. The ratio of this area to the total testicular area, expressed as a percentage, is used in the paper to designate the amount of interstitial tissue present in the specimens studied.

Non-syphilitic rabbits. The testes of two adult rabbits, never infected with syphilis, were studied for comparison. One of these rabbits was 2 years of age when killed. It had received no treatment of any kind (fig. 1). The other had been treated, from the age of one month, over a period of 15 months with from 3 to 15 R.U. (equivalent to 10–50 I.U. of estrone) of estrogenic substance daily (fig. 2).

RESULTS

Estrinized rabbits infected with syphilis. The histological alterations brought about by the action of the estrogens were always pronounced, irrespective of the presence or absence of syphilitic changes in the testis (fig. 3, 4). The degree to which the estrogens affected the size of the testis is shown particularly well by the weights obtained at the conclusion of the period of observation and treatment. The mean total weight of

the testes of an untreated rabbit was 1002.37 ± 84.0 mg./kg., and of an estrinized rabbit, 303.55 ± 30.86 mg./kg. An analysis of the weights of the testes affected by syphilis as compared with those in which no syphilitic lesion was demonstrable, showed that the sex hormones were the chief factors causing a loss of testicular substance (1). The reduction in weight, as shown by histological examination, was due to degenerative changes in the germinal epithelium causing a marked loss of spermatogenic cells. Such changes were usually homogeneous throughout the section. They were present in both testes of the estrinized animals, and were independent of the presence of a syphilitic process.

In spite of the extensive degeneration of the germinal epithelium, the general architecture of the testis was well preserved. There was sharp demarcation of the germinal from the interstitial tissues. The thickening of the tubular walls, due to an increase of connective tissue, was usually considerable, the thickness often being 2 or 3 times that found in the normal testis.

The degeneration of the germinal epithelium was characterized by the absence of spermatozoa and spermatids, and by a great decrease in the number of spermatocytes and spermatogonia. In many tubules, all that remained of the germinal cells was a group of lightly stained large cellular elements which were interpreted as Sertoli cells.

The amount of interstitial tissue was apparently increased throughout all of the sections examined, the area occupied being approximately equal to that of the tubules. Studying the interstitial tissue with the help of Mallory's connective tissue stain, it was found that the increase in connective tissue cells was general throughout the testis, but that the Leydig cells were increased only in places. Between the two types of cells (connective tissue and Leydig cells), there was a series of intermediate forms closely related morphologically, which suggested that the cells of Leydig may be primarily derived from connective tissue cells.

In only 3 of the 10 estrinized rabbits were there anatomical abnormalities of the testes which could with a fair degree of certainty be attributed to syphilis. With one exception, the syphilitic lesions were scattered focal collections of round cells (fig. 4). In one rabbit the testes, in addition, contained areas of vascularized scar tissue in which the tubular structures had entirely disappeared. In foci where both infection and estrinization had acted to change the picture, the extent of the damage was beyond that of either process alone (fig. 4).

Estrinized non-syphilitic rabbit. In order to define more clearly the characteristics of the tissue changes induced on the one hand by estrogenic action and on the other by syphilis, the testes from a rabbit treated for 15 months with daily injections of estrogenic substance were examined. The extensive degeneration of the seminiferous tubules and the relative increase in interstitial tissue corresponded to the changes found in the testes of the estrinized syphilitic rabbits, only they were more pronounced. This was due probably to the fact that estrogenic treatment began earlier in life, before the descent of the testes, and continued for a longer period of time. It may be concluded, therefore, that the alterations in structure observed in the estrinized rabbits infected with syphilis were the result chiefly of estrogenic action. This conclusion is supported also by the observations on the testes of the syphilitic estrinized rabbits that did not develop signs of orchitis.

Non-estrinized rabbits infected with syphilis. In the testes of some of the sexually intact syphilitic rabbits, microscopic evidence of orchitis was not conspicuous. This was particularly true in those cases where the orchitis, as judged from the clinical examination, had been mild and of short duration. In 2 animals that showed evidence of orchitis of both testes at some time during the course of the disease, only question-

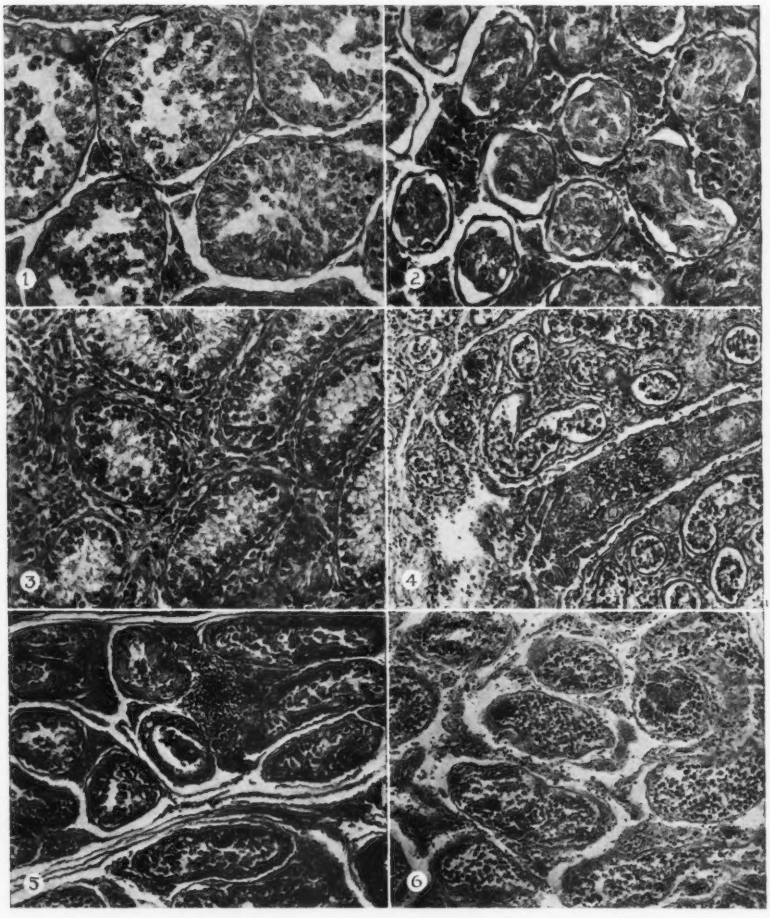


Fig. 1. Testis of normal adult rabbit. Mallory's stain. ×150. Fig. 2. Testis of normal rabbit treated with estrogens for 15 months, almost complete absence of germinal epithelium, and relative increase of interstitial tissue. Mallory's stain. ×150. Fig. 3. Testis of estrinized rabbit infected with syphilis. Atrophy of germinal epithelium. No evidence of orchitis. ×150. Fig. 4. Testis of rabbit treated with estrogens showing foci of inflammation due to syphilis. Compare with figure 5. H. and E. stain. ×58. Fig. 5. Testis of syphilitic non-estrinized rabbit showing degeneration of germinal epithelium and localized infiltration of interstitial tissue with inflammatory cells. H. and E. stain. ×78. Fig. 6. Testis of syphilitic non-estrinized rabbit with degeneration of germinal epithelium and diffuse inflammation, especially of the tubules. H. and E. stain. ×78.

able signs of inflammation were found. In one rabbit, on the other hand, there was microscopic evidence of orchitis of the uninoculated testis without there having been any manifest sign of a lesion during life. The lesions in this instance were limited to small localized areas of lymphocytic and plasma cell infiltration in the interstitial and germinal tissues.

At the other extreme, there were extensive and well-defined inflammatory and degenerative changes. These included hyalinization of the germinal epithelium and an

increase in the amount of interstitial tissue. In the interstitial spaces, the increase in tissue was the result of newly formed connective tissue cells. Between these limits, there were various gradations of inflammation and degeneration.

When the lesions were extensive and advanced, the general architecture of the testis was disturbed, the tubules being poorly differentiated from the interstitial tissues. The walls of the tubules were appreciably thickened in some places, and the epithelium was involved in a fairly dense lymphocytic reaction. There was arrest of spermatogenesis, and extensive degeneration of germinal epithelium (fig. 5). Partial, or complete, hyalinization was also present. Syncytial cells were not infrequently seen inside the partially degenerated tubules. In addition, there was an apparent increase in the number of cells and in the amount of connective tissue between the tubules (fig. 6). Under such circumstances, if the examination was confined to a localized area of the testis, there was some difficulty in deciding whether the tissue in question came from an estrinized or a non-estrinized animal, for the histological pictures presented much the same appearance. There was, however, one definite and clearly differentiating characteristic. In the non-estrinized animals, the degeneration was focal in character, whereas in the estrinized animals degeneration was uniform throughout the testis irrespective of the localization of the syphilitic process. In a testis from a non-estrinized rabbit, parts of the same section showed active spermatogenesis; this was never observed in the testes of animals treated with estrogenic substance.

Effect of estrogens on ratio of germinal to interstitial tissue of testis. One of the effects of the administration of estrogenic substance was the progressive decrease in the size of the testis. As determined by the weight of the organs, the loss of testicular substance was approximately 70% (1). From inspection of the histological preparations it looked as if the decrease in weight was the result of the loss of germinal epithelium. Although there was an apparent increase in the amount of interstitial tissue it seemed likely that this was only relative, due to the shrinkage in volume of tubular tissues with compression of interstitial cells into larger groups. In order to determine the degree to which the testicular components had been affected by treatment, sections of testis were measured by the technic previously described.

From the normal rabbits, 48 tracings were measured, and from the estrinized rabbits, 61 tracings. In the former group, the mean percentage of interstitial tissue was 28.71 ± 1.06, and in the estrinized group, 46.13 ± 0.99.[1] The critical ratio of the difference (17.42 ± 1.45) is 12.01, a highly significant variation.

If, however, these values are applied to the actual weights of the testes, which have already been recorded, it is seen that even though the ratio of interstitial tissue was significantly increased by estrogenic treatment, the actual weight of such tissue in the treated rabbit was much less than in the untreated rabbit. The testes of an animal in the latter group contained, on the average, 287.78 mg./kg. of interstitial tissue, while that of the estrinized rabbit contained only 140.03 mg.; a loss of 147.75 mg./kg.

An attempt to make actual counts of the Leydig cells in the sections was found impracticable. It is our impression, however, on the basis of the mathematical computations, that no actual increase in the number of these cells occurred, although it is possible that in some localized areas there was actual cellular proliferation.

This conclusion, while conforming in general to previous observations on the effect of estrogenic hormones, is at variance with other observations describing an increase in the interstitial cells following prolonged administration of estrogenic substance. Moore and Price (4), from a study of the reciprocal relationship between the

[1] Standard deviation of the mean is used throughout the paper.

gonads and hypophysis of rats, concluded that estrogenic hormones act to depress the functional activity of the testis, affecting the production of germ cells and the secretion of hormones alike. This effect they attributed to inhibition of the sex-stimulating secretion of the anterior pituitary gland, a conclusion which supported the earlier observations of Smith and Engle (5) and of Zondek and Ascheim (6), who demonstrated the vital role exerted by the anterior pituitary on spermatogenesis and the development of the testis.

Recent experiments by Burrows (7) describe an increase of interstitial cells in mice subjected to prolonged treatment with a pure estrogenic substance. This effect, however, is not constant, according to Gardner (8), even in animals of the same species. Furthermore, in those strains of mice showing the greatest interstitial hypertrophy, not enough hormone was produced by the testes to maintain the normal structure of the accessory sexual organs.

It may be assumed from the foregoing observations that an increase in the functional capacity of the glandular cells of the testis, following estrogenic treatment, has not been demonstrated. In fact one may with propriety assume a depression of the hormonal function under such circumstances. The alterations in the seminal vesicles, prostate and penis of the rabbits treated with estrogenic substance in our experiments would support this conclusion.

DISCUSSION

From a comparison of the histological changes induced in the testis by urinary estrogens with those caused by syphilis, it is apparent that both agents affect in general the same cellular elements, namely, the germinal epithelium of the seminiferous tubules. The only morphological differences observed were essentially quantitative, the estrogens being the more potent of the two agents. In animals subjected to prolonged estrogenic treatment, spermatogenic function was almost completely suppressed. Not infrequently, the entire series of spermatogenic cells were absent, leaving only the supporting cells of Sertoli. Testes so affected offered increased resistance to *Treponema pallidum*, and frequently did not react to the presence of this organism with either gross or microscopic changes of structure. It was also apparent that the extent to which the testis was affected by estrogenic action conditioned the degree of response to infection; the greater the depression of cellular activity, the milder the orchitis.

In the testis of the estrinized rabbit, there was also an apparent increase in the amount of interstitial tissue; the area occupied being approximately equal to that of the seminiferous tubules. In the normal rabbit the interstitial tissue makes up but a fourth to a third of the testis. However, calculation of the actual amount of interstitial tissue in the testes of the estrinized rabbit on the basis of the observed testicular weights, showed about 51% less interstitial substance than in the sexually intact rabbit. For this reason, unless an alteration in the functional capacity of the glandular cells of the testis can subsequently be demonstrated, we are inclined to believe that the germinal epithelium was the tissue of primary importance to the modification of the disease in the testes of the estrinized rabbits.

It seems reasonable to conclude, therefore, that the germinal epithelium constitutes a medium favorable to the growth of *Treponema pallidum*, or at least that these cells contain, or elaborate, some substance which acts to enhance the disease producing property of the organisms. When, by the influence of estrogenic substance, the normal biological processes of the germinal epithelium are depressed, and large numbers of cells completely destroyed, the treponemes may no longer excite an inflammatory response in the testis. Whether or not the increased resistance acquired

through depression of cellular activity in the testis was also effective in modifying the generalized reaction to infection remains a question.

The demonstration of the relationship of the germinal epithelium of the testis to the growth of *Treponema pallidum* is not without precedent. It has long been recognized that the testis of the rabbit is a favorable site in which to set up a syphilitic disease, whether from direct inoculation of virus or from its metastatic localization. As compared with the superficial layers of the skin of the rabbit, Chesney (9) found that the testis was a much more favorable site for the active proliferation of treponemata; and that rabbits inoculated intradermally were less prone to develop generalized lesions than those inoculated by way of the testis.

In subsequent studies, the same investigator (10, 11) observed that a granulating wound of the skin, or a coal tar dermatitis, including the fresh scars of the former, provided favorable foci for the lodgement and growth of *Treponema pallidum*. In connection with the response of the coal tar lesion to infection, attention was called to the remarkable proliferation of the epithelial cells of the skin, and it was pointed out that the two kinds of cutaneous lesions (granulating wound and coal tar dermatitis) were highly favorable to cell growth. It was thought possible that the factors which operate in the human body to bring about this rapid growth may exert a favorable effect upon the multiplication of *Treponema pallidum*.

Whatever the modus operandi may have been by which the effects of treatment were achieved, it was apparent that *Treponema pallidum* destroyed the same cellular elements in the testis as the estrogenic substance. When, by the administration of the hormones, the normal processes of growth in the germinal tissues were inhibited, or entirely suppressed, the testis tended to become refractory to syphilitic infection, and the disease in general to pursue a milder course. Whether factors other than the suppression of cellular activity in the germinal epithelium of the testis helped to modify the severity of the disease by affecting the processes of immunity in general must await further study.

SUMMARY

A study was made of the histological changes in the testes of rabbits subjected to prolonged treatment with estrogenic substance and subsequently infected with *Treponema pallidum*. Estrinizatin and infection were shown to depress the growth of the same cellular structure, namely, the germinal epithelium. When by estrogenic action the functional activity of the germinal cells was depressed, or completely arrested, the testis, in most instances, became refractory to syphilitic infection, and showed only slight or no alteration in structure attributable to this disease.

REFERENCES

1. Frazier, C. N., and C. K. Hu: *Endocrinology* 28: 283. 1941.
2. Frazier, C. N., and C. K. Hu: *Endocrinology* 28: 294. 1941.
3. Hu, C. K., and C. N. Frazier: *Proc. Soc. Exper. Biol. & Med.* 38: 116. 1938.
4. Moore, C. R., and D. Price: *Am. J. Anat.* 50: 13. 1932.
5. Smith, P. E., and E. T. Engle: *Am. J. Anat.* 40: 159. 1927.
6. Zondek, B., and S. Ascheim: *Arch. f. Gynäk.* 130: 1. 1927.
7. Burrows, H.: *J. Path. & Bact.* 42: 161. 1936.
8. Gardner, W. U.: *Anat. Rec.* 68: 339. 1937.
9. Chesney, A. M.: *J. Exper. Med.* 38: 627. 1923.
10. Chesney, A. M., and J. E. Kemp: *J. Exper. Med.* 41: 487. 1925.
11. Chesney, A. M., T. B. Turner and C. R. L. Halley: *Bull. Johns Hopkins Hosp.* 42: 319. 1928.

INFLUENCE OF ESTRONE ON THE ELECTRIC CHARACTERISTICS AND THE MOTILITY OF UTERINE MUSCLE

EMIL BOZLER

From the Department of Physiology, The Ohio State University
COLUMBUS, OHIO

THE CHARACTER of the movements of the uterus, in situ and isolated, differs strikingly in different phases of the sexual cycle, suggesting some hormonal influence on essential properties of uterine muscle. In the present paper the effect of estrone on this type of muscle will be investigated and it will be shown that changes in motility can be explained as the result of changes in muscular excitability.

METHODS

Uterine strips from the cat were prepared and mounted as described previously (1). The zinc-zinc sulfate electrodes used for these measurements made contact with the tissue by cotton wicks which were placed on the muscle at a distance of 3 cm. from each other. The potential change produced by treating the region around one of the leads with a 1% solution of KCl was taken to represent the resting potential. This potential determined at room temperature differed in strips from the same animal by as much as 25%, but it varied within still wider limits in different animals, generally being larger in larger animals. Only cats weighing 2.5 to 3 kg. were used.

For observation of the uterus in situ, rabbits anesthetized with urethane and cats anesthetized with nembutal were used. The exposed uterus was protected against drying and cooling by a thick glass cover.

Because activity produces potential changes and wide fluctuations in excitability, only perfectly quiescent preparations can be used. Rodents were not found to be suitable for this work because preparations from these animals usually show some movements. On the contrary, strips from the cat's uterus suspended in air were spontaneously active only if the animal had received injections of theelin for at least 5 days and even in these preparations there usually were long periods of complete quiescence during which measurements could be made.

RESULTS

In experiments, performed outside the normal breeding season, cats received daily injections of theelin (500 I.U. in oil intraperitoneally). The electric excitability of uterine strips from these animals was determined by measuring the rheobase. During anestrus the uterus was non-excitable for electric stimuli as reported previously (1). Following the administration of theelin the electric excitability gradually increased (fig. 1A).

In the rat's uterus Katzenstein (4) has observed a variation in the chronaxie during the sexual cycle. This result cannot be correlated with the observations reported in this paper because chronaxie measures only the time factor of excitability, not excitability itself, and because both of these characteristics are not closely related to each other.

Also the character of the response to electric stimuli was altered. In the highly excitable strips obtained by treatment for 4 to 5 days the contraction produced by a a single shock travelled over the whole muscle at a speed of 4 to 5 cm. per second whereas in preparations from animals treated for a shorter period of time the response was confined to the region of the cathode. Reynolds (7) made similar observations for the response to nerve stimulation in the rabbit's uterus.

The injections of theelin, furthermore, increased the resting potential (fig. 1B). This effect can partly be explained by the hypertrophy of the musculature, but only if it is assumed that the muscle cells enlarge more than the interspaces and other constituents of the organ. Actually, however, administration of the hormone also pro-

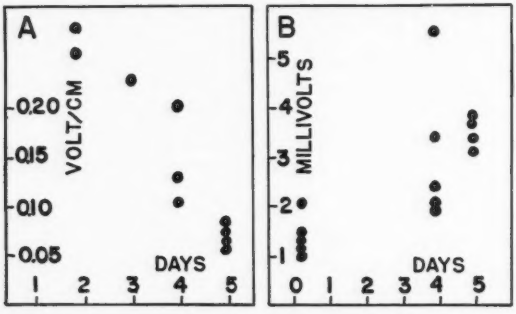


Fig. 1. Effect of injections of theelin on the excitability and resting potential of cat uterine muscle. Each point represents the average of measurements on several strips from the same animal. A. variation of rheobase, expressed in volt per cm. distance of the stimulating electrodes. Temp. 34 to 35°. B. variation of the resting potential as determined by applying 1% KCl on one of the electrodes. Room temperature.

duces fibrocytic hyperplasia, edema and increased vascularity (cf. 6), conditions which will tend to diminish the observed effect. Quantitative data on these changes are not available and it is, therefore, not possible to evaluate their effect on the resting potential accurately. It seems, however, that the growth response of the uterus in the cat is much slower than the rodents and it seems improbable that the relative increase in the size of the muscle fibers, if present, be large enough to explain the 2- to 3-fold increase in the resting potential. It is more likely therefore, that this effect is due to some alteration of cellular activity. Possibly it results from a change in the distribution of electrolytes in the tissue, but no data on this question are available. The increase in resting potential by estrone is probably related to the increase in electric excitability just described and to the elevation of resting metabolism (cf. 6). All these changes may be considered as manifestations of the transformation of uterine muscle from a dormant state into one of full activity.

The change in muscular excitability produced by estrone provides an explanation for the differences in the character of the uterine movements during estrus and anestrus. During anestrus muscular movement is weak and incoordinated (cf. 6). Close observation of the organ under this condition, isolated or in situ, reveals that contractions originate independently in various regions and that the activity remains localized. This incoordination undoubtedly results from the low excitability and the resulting failure of conduction of muscular impulses.

During estrus one can clearly distinguish two types of movements [Pompen (5) and our own observations]. Following a period of quiescence a wave of contraction

sweeps over the whole uterus at a rate estimated at 3 to 4 cm. a second. The activity initiated by this rapid wave lasts for different periods in different parts of the uterus. In the rabbit the regions which remain active longest appear as deeply constricted rings. These rings are stationary or they travel very slowly (2 mm. per second or less). New waves of contraction usually originate at these constrictions and travel for some distance, often as far as the nearest contraction ring. This type of activity may persist for 20 to 30 seconds, after which a period of complete rest intervenes.

Electrophysiological studies have thrown some light on the nature of these movements. They are accompanied by a repetitive discharge of impulses conducted within the syncytial musculature and may, therefore, be considered as tetanic contractions (2). These findings explain the occurrence, in the same organ, of rapid and slow waves of contraction. The rapid waves resemble the peristaltic rush of the intestine (3) and probably are due to the conduction of a train of impulses over the entire musculature. The rate of conduction of this process agrees well with that of single impulses in isolated uterine muscle. Because this process occurs only during a state of high excitability it becomes intelligible that the rapid waves are observed only after a period of inactivity. Contraction rings probably represent a persistent tetanic contraction. They are formed after previous activity when the muscle is in a state of low excitability and the conduction of impulses, consequently, is limited. This type of activity is more localized and its propagation is determined by the gradual return of excitability in various regions of the organ.

There are great differences in the rate of conduction of impulses and other characteristics in various species. By electrophysiological methods (2) the rate of conduction during estrus was found to be about 6 cm. per second in the cat, 4 cm. per second in the rabbit, and 1 to 2 cm. per second in the guinea pig. The chronaxies vary correspondingly, being shortest in the cat and longest in the guinea pig (1). These differences probably explain, at least partly, the differences in the character of motility. In agreement with the faster conduction, the contractions of the whole uterus are more synchronous in the cat than in the other species.

SUMMARY

Injections of estrone produce an increase in electric excitability and in the resting potential in the cat's uterus.

Responses of the isolated uterus to electric shocks are local during anestrus, but conducted during estrus. These differences, which are due to changes in excitability, explain why the motility of the uterus is different during estrus and anestrus.

Rapidly conducted contraction waves and slowly moving contraction rings are explained as due to the conduction of muscular impulses.

I am indebted to Dr. S. R. M. Reynolds for reading the manuscript and for valuable suggestions.

REFERENCES

1. Bozler, E.: *Am. J. Physiol.* 122: 614. 1938.
2. Bozler, E.: *Am. J. Physiol.* 124: 504. 1938.
3. Bozler, E.: *Am. J. Physiol.* 127: 301. 1939.
4. Katzenstein, F. C.: *Endocrinology* 22: 579. 1938.
5. Pompen, A. W. M.: Thesis, Amsterdam, 1933.
6. Reynolds, S. R. M.: The physiology of the uterus, 1939.
7. Reynolds, S. R. M.: *Am. J. Physiol.* 112: 640. 1935.

HORMONAL CONTENT OF THE PHARYNGEAL PITUITARY GLAND

STEPHEN TOLINS AND ROBERT A. MOORE

From the Department of Pathology of the New York Hospital and Cornell University Medical College, and of the Washington University School of Medicine

NEW YORK CITY AND ST. LOUIS, MISSOURI

THAT there remains a small piece of pituitary tissue in the pharyngeal mucosa, designated as the pharyngeal pituitary gland, has been demonstrated (1, 2). Melchionna and Moore (3) proved its presence in 51 out of 54 cases studied. All of these authors discussed a possible physiological function of the pharyngeal pituitary gland, but came to no definite conclusions. Haberfeld (1) reported no change in the pharyngeal pituitary of five pregnant women. Melchionna and Moore (3) observed only epithelial cells in the pharyngeal pituitary of a pregnant woman. Christeller (2) reported two cases of compression of the pituitary by tumor in which there was no compensatory hypertrophy of the pharyngeal pituitary. Indicative of possible activity are the cases of Christeller and of Melchionna and Moore in which prominent eosinophilic cells in the pharyngeal pituitary were associated with Fröhlich's syndrome and with teratoma of the testis.

The present study was undertaken to determine directly whether or not the pharyngeal pituitary contains hormones similar to those in the pituitary gland. The possible presence of the gonadotropic, thyrotropic and adrenotropic hormones was investigated.

METHOD

The pharyngeal pituitaries were obtained from autopsies at the New York Hospital. Those from adults without evidence of hormonal dysfunction were used. No other attempt at selection was made. The patients ranged in age from 38 to 71 years, with an average age of 49 years.

The base of the skull was exposed in the routine fashion. A coronal cut with a chisel was then made anterior to the optic groove and extended into the posterior nasopharynx. Sawcuts through the thickness of the base of the skull were extended from each lateral extremity of the chisel cut, and made to meet in the occipital bone just anterior to the foramen magnum. The pharyngeal pituitary is a small structure and it is not feasible to dissect it free from the mucosa. It is, however, always located in the midline directly over the vomerosphenoidal articulation. A block of tissue 5×15 mm. was therefore cut from the midline of the mucosa of the nasopharynx overlying the vomer and vomero-sphenoidal articulation and used for implantation. This method for obtaining the pharyngeal pituitary is described in greater detail by Melchionna and Moore. A second piece of pharyngeal mucosa of similar size was cut from each case at some distance from the site of the pharyngeal pituitary to serve as a control.

The hormonal content of these pieces of tissue was determined by implantation

Received for publication April 28, 1941.

into immature female guinea pigs according to the technic of Kunkel and Loeb (4). One-quarter of the total amount of tissue was implanted subcutaneously each day for 4 days and the animal killed on the fifth day. The ovaries, adrenals and thyroid were removed for histological study. The guinea pigs had an initial body weight of 130 to 160 gm. and did not exceed 190 gm. at the time they were killed.

Two guinea pigs were implanted with a total of one pharyngeal pituitary, 2 with a total of 2, 2 with a total of 3, and one with a total of 4. A similar number of controls were used. Four normal guinea pigs, not implanted were studied as a further control.

In order to determine the least weight of pituitary tissue required to produce a response in the guinea pig, the anterior lobe of the pituitary of 5 cases was implanted so that a total dose of 5, 10, 25, 30, 40 and 50 mg. was secured over 4 days. These animals were treated and studied as were the other guinea pigs.

RESULTS AND DISCUSSION

Kunkel and Loeb have described the effects of human anterior pituitary on the ovaries, thyroid and adrenal of the guinea pig. The growth, maturation and leuteinization of the follicles in the ovaries, the hypertrophy of the thryoid, and the increased proliferative activity in the adrenal which they described, were seen in none of the guinea pigs implanted with the pharyngeal pituitary. The control animals, implanted with pharyngeal mucosa, likewise yielded negative results. The changes described above, however, were noted in the guinea pigs implanted with as little as 10 mg. of the anterior lobe of the pituitary gland, which was a smaller piece of tissue than a total of three pharyngeal pituitaries. The anterior pituitary, from 5 of the cases which provided the pharyngeal pituitaries were used so that there was no age discrepancy.

CONCLUSION

The pharyngeal pituitary does not contain the gonadotropic, adrenotropic or thyrotropic hormones in an amount which is contained in 10 mg. of the anterior lobe of the pituitary.

REFERENCES

1. Haberfeld, W.: *Beitr. z. path. Anat. u. z. allg. Path.* 46: 133. 1909.
2. Christeller, E.: *Arch. f. path. Anat.* 218: 185. 1914.
3. Melchionna, R. H., and R. A. Moore: *Am. J. Path.* 14: 763. 1938.
4. Kunkel, P., and L. Loeb: *Proc. Soc. Exper. Biol. & Med.* 32: 1413. 1935.

CHRONIC TOXICITY STUDIES OF DIETHYL-STILBESTROL

II. SUBCUTANEOUS IMPLANTATION OF PELLETS IN RATS

R. C. PAGE, H. K. RUSSELL, E. L. SCHWABE, C. S. MATTHEWS AND F. E. EMERY

From St. Agnes Hospital and the Departments of Physiology and Materia Medica, University of Buffalo

WHITE PLAINS AND BUFFALO, NEW YORK

TOXICITY STUDIES of stilbestrol are of importance because of the many divergent reports in the current medical literature from both clinical and experimental investigators. In a previous report by the present authors (1) stilbestrol was given orally to rats and histological study of the organs and tissues was performed. This mode of administration is questionable because of variable absorption of the drug from the gastrointestinal tract and because of the rapidity of detoxification in the liver following the absorption of the drug into the portal circulation.

In the toxicity studies of stilbestrol we have given the drug in sesame oil subcutaneously, intramuscularly and intraperitoneally to other groups of rats. This mode of administration was not considered satisfactory for chronic toxicity studies because oil cysts were formed at the site of injection in many of the animals. These cysts persisted for long periods and on removal were found by assay to contain varying proportions of the originally injected stilbestrol (2).

Another method of obtaining effect from stilbestrol is the subcutaneous implantation of firmly compressed pellets so that it is possible to determine the actual amount of stilbestrol absorbed; it is reasonable to assume that it has entered the tissues during the specific period of time that the pellet remains implanted.

METHODS

Stilbestrol pellets (50 and 200 mg.) were specially compressed at an estimated pressure of 350 lb. per square inch.[1] One weighed pellet was implanted subcutaneously with aseptic precautions in each rat, usually in the shoulder or flank.

Normal and castrated adult albino female rats, of Wistar stock inbred for many generations, were used in these experiments. Vaginal smears were taken daily and recorded and will be reported in a separate paper. The animals were killed at varying intervals in order that the tissue changes could be studied in relation to the various amounts of stilbestrol absorbed. After each animal was killed the pellet was removed, dried and weighed. A complete autopsy was performed on each animal. Bone marrow and peripheral blood smears were made and the pituitary, adrenals, ovaries, liver, kidneys, spleen and uterus of most of the animals were weighed and saved for histologic study. Tissues of untreated normal and castrate females of comparable age were used for comparison.

RESULTS

Group I, 50 mg. stilbestrol pellets, normal female rats. Pellets were implanted subcutaneously in 10 animals for 19 to 56 days. Gross and histologic findings are outlined

Received for publication May 10, 1941.

[1] The stilbestrol was supplied by Burroughs Wellcome & Co., Inc., New York City.

in table 1. (In tables 1, 2, 3 and 4, only positive findings are recorded. Inclusion of negative data would make this report unduly lengthy.) The weights of the animals remained almost stationary during the experiment (table 5). The average amount of stilbestrol absorbed daily was 0.55 mg. (table 5). Significant changes in organ weights were shown by a decrease in ovarian weight and an increase in weights of the adrenals (table 6).

Group II, *50 mg. stilbestrol pellets, castrate female rats.* Pellets were implanted subcutaneously in 10 animals for 10 to 116 days. Pathologic changes are outlined in

TABLE 1. GROUP I, EFFECTS OF 50 MG. STILBESTROL PELLETS ON NORMAL FEMALE RATS

Rat No.	Stilbestrol		Gross findings at autopsy	Histologic study of tissues
	Days implanted	Amt. absorbed		
1	37	Lost on 37th day	Ovaries hemorrhagic, uterine horns enlarged	Ovary: multiple abscesses, endometrial hyperplasia, blood: platelets diminished
2	24	Lost on 24th day	Uterine horns distended	Myosarcoma of uterus, ovary: large corpora lutea
3	19	8 mg.	Uterine horns enlarged	Endometrial hyperplasia, pyometra
4	24	Lost on 24th day	Negative	Liver: fatty degeneration; endometrial hyperplasia
5	24	Lost on 23rd day	Left uterine horn enlarged	Pyometra, endometrial hyerplasia
6	25	15 mg.	Negative	Endometrial hyperplasia
7	25	12 mg.	Uterine horns distended, spleen enlarged	Abscess of uterine horn (pyometra), endometrial hyperplasia, bone marrow: neutrophilic hyperplasia, blood: neutrophilic leucocytosis
8	25	15 mg.	Negative	Endometrial hyperplasia
9	56	27.6 mg.	Adrenals hemorrhagic, ovaries atrophic, uterine horns enlarged	Adrenals: small areas of cortical hemorrhage and few necrotic cells; pyometra, mammary gland: cystic dilatation of ducts and glands
10	46	28 mg.	Adrenals reddish-brown	Adrenals: hemorrhage in cortex; ovaries: atrophic; chronic cystic mastitis; uterus: hyperplasia, endometritis (pyometra)

table 2. The animals lost 12% of their original weight during the course of the experiment (table 5). The average amount of stilbestrol absorbed daily was 0.42 mg. (table 5) which is slightly less than that of the normal animals. The most significant organ weight change was found in the pituitary glands of two of the animals that had pellets implanted for 116 days; they weighed 222.9 mg. and 170 mg. respectively and both showed marked enlargement of the anterior lobes (table 6).

Group III, *200 mg. stilbestrol pellets, normal female rats.* Pellets were implanted subcutaneously in 10 animals for 23 to 31 days. Gross and histologic findings are outlined in table 3. The animals lost about 25% of their original weight (table 5). The average amount of stilbestrol absorbed daily was 1.88 mg. (table 5). Organ weights

TABLE 2. GROUP II, EFFECTS OF 50 MG. STILBESTROL PELLETS ON CASTRATE FEMALE RATS

Rat No.	Stilbestrol		Gross findings at autopsy	Histologic study of tissues
	Days implanted	Amt. absorbed		
		mg.		
1	10	15	Negative	Endometrial hyperplasia; liver: fatty degeneration (very slight); pituitary: hyperplasia of chromophobe cells
2	10	7.6	Tumor mass of left kidney	Adrenal abscess and cortical hemorrhage; endometrial hyperplasia, pyometra; kidney: abscess; liver: fatty degeneration; blood: neutrophilic leucocytosis
3	61	45.9	Negative	Endometrium: squamous metaplasia; liver: early toxic necrosis in rare scattered cell (fat stain negative)
4	116	38.7	Animal emaciated, left horn of uterus enlarged; pituitary: markedly enlarged, soft and bulging out of sella turcica	Endometritis (pyometra); pituitary: hyperplasia of chromophobe cells
5	116	33	Uterus enlarged; pituitary: markedly enlarged and hemorrhagic and bulging out of sella turcica	Pyometra; endometrium: squamous metaplasia; pituitary: hyperplasia of chromophobe cells
6	116	41.4	Uterus enlarged	Endometrial hyperplasia; pituitary: hyperplasia of chromophobe cells

TABLE 3. GROUP III, EFFECTS OF 200 MG. STILBESTROL PELLETS ON NORMAL FEMALE RATS

Rat No.	Stilbestrol		Gross findings at autopsy	Histologic study of tissues
	Days implanted	Amt. absorbed		
		mg.		
1	25	Lost on 25th day	Negative	Liver: areas of cloudy swelling, ovaries: follicular hyperplasia
2	23	40	Negative	Liver: cloudy swelling; chronic cystic mastitis; ovary: large corpora lutea
3	25	46.5	Uterine horns distended	Ovaries atrophic; kidneys: areas of tubular degeneration; liver: focal areas of toxic necrosis; pituitary: congestion of anterior lobe
4	31	36	Uterus enlarged and filled with serous fluid; spleen: enlarged and studded with punctate gray areas; adrenals hemorrhagic	Pyometra; adrenals: areas of hemorrhage throughout glands; spleen: areas of focal necrosis; ovaries: atrophic
5	25	70	Enlarged uterus	Ovaries: atrophic; pyometra; pituitary: hyperplasia of chromophobe cells

TABLE 4. GROUP IV, EFFECTS OF 200 MG. STILBESTROL PELLETS ON CASTRATE FEMALE RATS

Rat No.	Stilbestrol		Gross findings at autopsy	Histologic study of tissues
	Days implanted	Amt. absorbed		
		mg.		
1	49	53.5	Adrenals hemorrhagic	Few areas of hemorrhage in adrenal cortex; pituitary: hyperplasia of chromophobe cells; pyometra; bone marrow: neutrophilic hyperplasia
2	34	39.6	Uterine horns distended; adrenals hemorrhagic	Pyometra; areas of hemorrhage in adrenal cortex
3	42	81	Enlarged pituitary	Pituitary: hyperplasia of chromophobe cells; chronic cystic mastitis
4	35	56.7	Uterine horns and pituitary enlarged	Adrenals: hemorrhage in zona reticularis; pituitary: hyperplasia of chromophobe cells; liver: early toxic changes (fat stain showed fatty infiltration); pyometra; bone marrow: neutrophilic hyperplasia
5	34	Lost on 34th day	Liver, adrenals and uterine horns enlarged; kidneys: hemorrhagic	Liver: focal areas of necrosis; kidney: areas of hemorrhage throughout; pituitary: anterior lobe, hyperplasia of chromophobe cells with hyperemia and small scattered hemorrhages; bone marrow: hypoplastic with only occasional megakaryocyte; cystic mastitis
6	27	35.2	Ascites; uterine horns enlarged; adrenals hemorrhagic	Adrenals: hemorrhage of zona reticularis; pyometra and parametritis
7	34	44	Adrenals hemorrhagic	Adrenals: hemorrhage in cortex; endometrium: hyperplasia
8	34	94.7	Pituitary enlarged	Hyperplasia of endometrium; anterior pituitary: hyperplasia of chromophobe cells and hyperemia
9	34	48.7	Adrenals hemorrhagic	Hemorrhage in adrenal cortex; early toxic necrosis of only a few scattered liver cells; hyperplasia of chromophobe cells and congestion of anterior pituitary
10	34	41.8	Adrenals hemorrhagic	Hemorrhage in adrenal cortex; hyperemia of anterior pituitary; pyometra; bone marrow: neutrophilic hyperplasia; blood smear: neutrophilic leucocytosis

recorded at autopsy are shown in table 6. The uteri were most affected, their average weight being three times that of the normal controls.

Group IV, *200 mg. stilbestrol pellets, castrate female rats.* Pellets were implanted subcutaneously in 16 animals and remained in situ for 27 to 49 days. Gross and histologic findings are shown in table 5. The animals lost about 16% of their original weight (table 5). The average amount of stilbestrol absorbed daily was 1.53 mg. (table 5). The pituitary glands were most affected, their average weight being three times that of the castrate controls (table 6).

TABLE 5. AVERAGE ABSORPTION OF STILBESTROL PELLETS AND EFFECT ON RAT WEIGHTS

Group	No. of rats	Av. days	Av. wt. at start of experiment	Av. wt. at end	Av. loss or gain	Av. amount stilbestrol absorbed	Av. daily absorption
			gm.	gm.	gm.	mg.	mg.
I 50 mg. Normal	10	35.4	178.0	179.2	+ 1.2	19.5	0.55
II 50 mg. Castrates	10	71.5	164.0	143.0	−21.0	30.3	0.42
III 200 mg. Normals	10	22.8	206.0	157.0	−49.0	42.9	1.88
IV 200 mg. Castrates	16	35.9	223.6	186.6	−37.0	55.02	1.53

Effect on Body Growth

Of the 4 groups that had stilbestrol pellets implanted only one group (Group I, table 1) maintained their weight to the end of the experiment (fig. 1). Generally the animals in which 50 mg. stilbestrol pellets had been implanted showed less loss of body weight than the animals in which 200 mg. stilbestrol pellets had been implanted. Where the larger pellets (200 mg.) had been implanted, absorption on the average was about three times as great as in the animals in which the 50 mg. pellets had been implanted.

Absorption of Stilbestrol from Pellets

The stilbestrol pellets were compressed under great pressure (350 lb. per square inch) so that they would not disintegrate or fracture while in the body tissues. An occasional pellet sloughed from the animals; however, in general this route of ad-

TABLE 6. EFFECT OF STILBESTROL IMPLANTED SUBCUTANEOUSLY ON ORGAN WEIGHTS OF FEMALE RATS

Group	Pituitary		Ovaries		Adrenals		Uterus		Spleen		Liver		Kidney	
	Av. wt. at autopsy	Wt. per 100 gm. body wt.	Av. wt. at autopsy	Wt. per 100 gm. body wt.	Av. wt. at autopsy	Wt. per 100 gm. body wt.	Av. wt. at autopsy	Wt. per 100 gm. body wt.	Av. wt. at autopsy	Wt. per 100 gm. body wt.	Av. wt. at autopsy	Wt. per 100 gm. body wt.	Av. wt. at autopsy	Wt. per 100 gm. body wt.
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
I 50 mg. pellets Normals	15.9	8.8	27.0	15.1	90.6	58.4								
II 50 mg. pellets Castrates	78.5	57.0[1]			51.9	38.1	507.4	373.0	493.1	379.0	6,090	4,684	1,667	1,287.0
III 200 mg. pellets Normals	14.5	10.7	38.1	28.0	60.3	44.3	899.9	661.0	704.0	517.0			1207	887.0
IV 200 mg. pellets Castrates	29.3	16.2			67.4	38.0	670.4	364.3						
Control organ weights per 100 gm. body weight[2]		5.3		33.1		31.6		217.0		566.7		3,786		799.2

[1] This figure is high because of the inclusion of 2 pituitaries weighing 222.9 and 170 mg. respectively from animals that went 116 days with stilbestrol pellets.

[2] These figures obtained from table 2 of Organ Weight Study by Emery, Emery and Schwabe (3) on same rat colony.

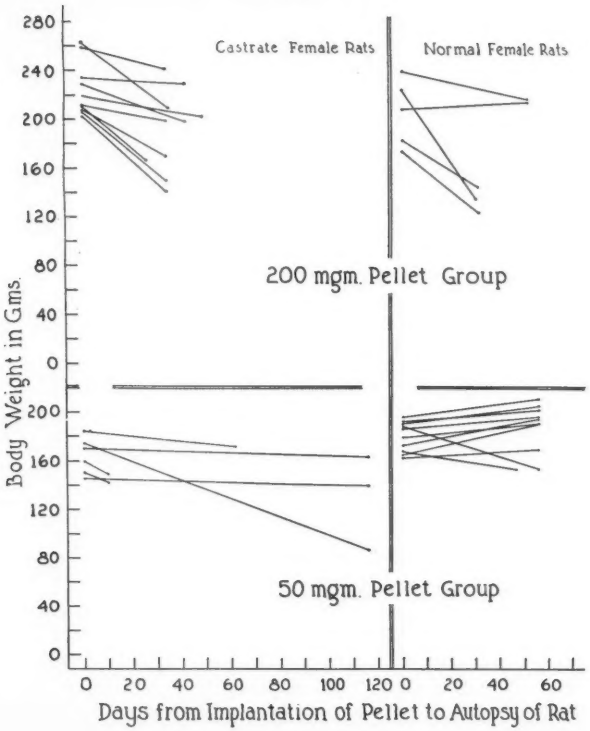

Castrate Female Rats
Normal Female Rats
200 mgm. Pellet Group
50 mgm. Pellet Group
Body Weight in Gms.
Days from Implantation of Pellet to Autopsy of Rat

Fig. 1. BODY WEIGHT DIFFERENCE FOLLOWING ABSORPTION OF STILBESTROL PELLET.

Fig. 2. 1a, 200 mg. stilbestrol pellet; 1b, pellets on removal from animals; 2a, 50 mg. stilbestrol pellet; 2b, pellets on removal from animals.

ministration has been quite satisfactory. A fibrous capsule is formed about the pellet after its implantation similar to that formed about a foreign body. As the fibrous capsule increases in thickness and compactness it is probable that absorption de-

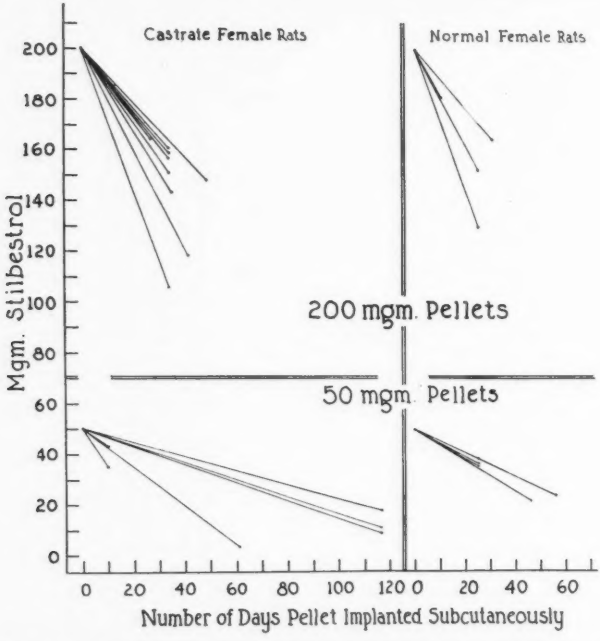


Fig. 3. ABSORPTION OF STILBESTROL FROM 50 AND 200 MG. PELLETS IN NORMAL AND CASTRATE FEMALE RATS.

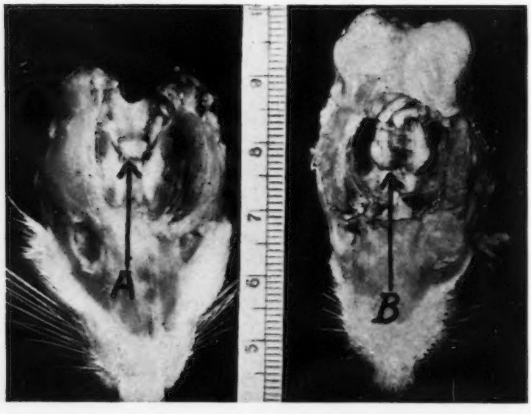


Fig. 4A. PITUITARY OF NORMAL FEMALE RAT, WT., 8.1 mg. Fig. 4B. PITUITARY OF CASTRATE *rat* 5 of Group II, table 2, wt., 170 mg.

creases in proportion. The absorption of stilbestrol appeared to be from the surface of the pellets which, on removal, were rough and pitted (fig. 2).

The average daily absorption of stilbestrol from the 50 mg. pellets was 0.55 mg. for the normal rats and 0.42 mg. for the castrate rats (table 5). From the 200 mg. pellets, the average daily absorption was 1.88 mg. for the normal rats and 1.53 mg. for the

castrate animals. Absorption from similar size pellets in castrate and normal female rats was approximately equal so that physiological need for estrogenic hormone apparently plays no part in absorption. Thus it may be assumed that the amount of stilbestrol absorbed from pellets implanted subcutaneously is proportional to the total surface area of the pellet (fig. 3).

There was an individual difference in absorption of stilbestrol in the same group of rats, e.g., *rat 8* (table 4) absorbed 94.7 mg. from a 200 mg. pellet in 34 days while *rat 10* absorbed only 41.8 mg. from the same size pellet in the same time. As the pellets

TABLE 7. EFFECTS OF STILBESTROL ON THE TYPE CELLS OF THE ANTERIOR PITUITARY

Normal female rats	Days pellet implanted	Stilbestrol absorbed	Wt. pit. per 100 gm. body wt.	Acidophil cells	Basophil cells	Chromophobe cells
		mg.	mg.	%	%	%
Controls	—	—	5.3	20	7	73
200 mg. pellet	35	64.2	21.6	12	2	86
200 mg. pellet	35	88.5	31.2	7	3	90
200 mg. pellet	35	18.0	28.7	16	2	82
50 mg. pellet	116	33.0	120.5	10	2	88

were all from the same batch it is probable that they were compressed to approximately the same degree of hardness. The rate of formation and compactness of the fibrous capsule found about the pellet probably is responsible in some measure for this varied amount of absorption.

MacBryde et al. (4) implanted 100 mg. pellets of stilbestrol dipropionate subcutaneously in castrate women. The response was marked in 7 to 10 days, with active proliferation of the endometrium within 7 days. Average daily absorption ranged from 0.127 to 0.25 mg. This study would indicate that the rats absorbed two to six times as much stilbestrol from the pellets as did the castrate women.

Effect on Anterior Pituitary

The estrogenic hormones have been shown to have a profound effect on the anterior lobe of the pituitary. An excess amount causes the pituitary to increase in weight (1, 5), causes an increase in the percentage of chromophobe cells, and a decrease in percentage of acidophil and basophil cells.

All of the animals in which pellets of stilbestrol had been implanted had enlarged pituitaries; however, the increased weight was greater in those of the castrate animals than in those of the normal animals (table 5). Two of the castrate females (*rat 4* and 5 of Group II, table 2) had enormous pituitaries that weighed 222.9 and 170 mg. respectively (fig. 4). In these animals stilbestrol was implanted for 116 days (the longest period), but they absorbed only 38.7 and 33 mg. respectively. Some of the animals in which 200 mg. pellets were implanted absorbed much more stilbestrol (94.7 mg. in 34 days) but none of these had such enormous pituitaries.

Wolfe's (6) technic was employed in making cell counts of pituitaries of normal and stilbestrol pellet animals. Table 7 shows that in the normal female rats there was an absolute increase in the percentage of chromophobe cells and a relative decrease in the percentage of acidophil and basophil cells in the stilbestrol treated animals.

Histological Findings

The main object of this study was to determine tissue changes caused by absorption of definitely large amounts of stilbesterol in rats, and the results of study of each animal as a unit are shown in tables 1, 2, 3 and 4.

Organs of 31 animals were studied histologically and livers of 10 showed definite pathologic change of which 5 had toxic necrosis, 3 fatty degeneration and 2 had cloudy swelling of hepatic cells.

The kidneys were resistant to exposure to large doses of stilbestrol. In 3 of the rats the kidneys showed toxic changes; one had areas of tubular degeneration, another had scattered areas of hemorrhage, and a third had an abscess (the causal relation of

TABLE 8. AVERAGE DAILY AMOUNT OF STILBESTROL ABSORBED FROM PELLETS COMPARED WITH MINIMAL AMOUNTS OF STILBESTROL REQUIRED TO BRING ADULT FEMALE CASTRATED RATS INTO ESTRUS

	Group I Normal rats, 50 mg. pellets	Group II Castrates 50 mg. pellets	Group III Normal rats, 200 mg. pellets	Group IV Castrates 200 mg. pellets
Av. daily absorption of stilbestrol	550γ	420γ	1,880γ	1,530γ
Minimal amounts of stilbestrol producing estrus[2]				
Dodds et al. (9) 0.25γ in alcohol aqueous solution, subcut.	2,200	1,640	7,520	6,120
Sondern & Sealey (10) 1.7γ in vegetable oil subcut. gave response in 50% of animals	323	247	1,106	900
Dodds et al. (11) 0.35γ in sesame oil subcut. gave response in 60% of animals	1,571	1,200	5,371	4,371
Matthews & Schwabe,[1] 0.30γ in sesame oil subcut. gave response in 100% of animals	1,833	1,400	6,266	5,100
Matthews & Schwabe,[1] 0.07γ in sesame oil subcut. gave response in 50% of animals	7,857	6,000	26,857	21,857
Matthews & Schwabe,[1] 0.017γ in dilute NaOH subcut. gave response in 100% of animals	32,353	24,705	110,588	90,000
Matthews & Schwabe,[1] 0.008γ in dilute NaOH subcut. gave response in 50% of animals	68,750	52,500	235,000	191,250

[1] Unpublished data on same rat colony used in this study.
[2] Values are given in the number of times the minimal dose of stilbestrol absorbed daily.

stilbestrol to the abscess in the animal is probably not a direct one inasmuch as the animal had a pyometra and leucocytosis).

The uterus being directly controlled by the ovarian hormones would be expected to respond markedly to huge doses of estrogenic substances. Such an effect was evidenced by endometrial hyperplasia in 13 animals, pyometra in 12, and squamous metaplasia of the endometrium in 2 animals. At autopsy a careful search was made for tumors, especially of the uterus, and the single uterine myosarcoma was the only one found.

Adrenals of 11 animals showed toxic manifestations, chiefly hemorrhage in the cortex. Mammary glands showed chronic cystic mastitis which is the usual result of continued estrogenic stimulation. Only the spleen of one animal showed pathologic change which consisted in areas of focal necrosis; this probably was related to the presence of a pyometra.

Study of peripheral blood smears made at the time of autopsy showed neutrophilic leucocytosis in 3 animals that had pyometra. In addition one animal showed a marked decrease in number of blood platelets.

Study of bone marrow smears showed neutrophilic hyperplasia in 4 animals that had pyometra and hypoplasia with marked decrease in number of megakaryocytes in one animal.

DISCUSSION

The animals in these experiments absorbed enormous amounts of stilbestrol when compared to the amount of stilbestrol injected subcutaneously that is necessary to cause estrus in castrate female rats (table 8).

The pathologic changes found in these animals were present as a direct consequence of marked overdosage of stilbestrol far beyond that required for normal physiologic need. This amount of stilbestrol was several thousand times the estrinizing dose (table 8). In view of the fact that less than one-third of the animals developed any evidence of toxicity it may be suggested that with smaller doses, nearer the physiologic requirement, the toxicity would be at a minimum or entirely absent.

It has been shown that stilbestrol is normally detoxified in the liver (7). Because of this fact it seems logical that the liver would be the organ most likely to show toxic changes in the presence of large amounts of stilbestrol such as were absorbed in the rats in this study.

The tumorigenic power of stilbestrol in castrate guinea pigs was evidenced with doses of 3 to 10γ stilbestrol in olive oil, given subcutaneously, weekly, for 87 to 115 days (8). This was manifest by fibrous scars on the uterine apex and on the uterine horns at junction of the uterine horn and mesometrium. Search was made for these abnormalities in our rats but no evidence of such tumorigenic power was present. It is probable that a species difference is responsible for such divergent findings and it would suggest that the rat is more resistant to the tumorigenic power of stilbestrol than is the guinea pig. The single uterine myosarcoma that was found was considered by us to be only of chance occurrence inasmuch as no other evidence of true tumor formation was present. The squamous metaplasia of the endometrium that was found in two of the animals can probably be explained on the basis of extension of the keratinized vaginal epithelium beyond its normal limits and into the endometrium.

Inhibition of body growth was present in all animals, and from these animals it may be assumed that the loss of body weight is roughly proportional to the amount of stilbestrol absorbed. The pituitary responded to stilbestrol by an increase in weight which was related more to the length of time that the stilbestrol was implanted than by the actual amount absorbed. Toxic changes were found in about one-third of the animals and were confined mainly to the liver, adrenals, pituitary and kidney with only a few animals showing changes in the hemopoietic system.

SUMMARY

From the studies reported we conclude that 50 and 200 mg. stilbestrol pellets implanted subcutaneously in both normal and castrate female rats allowed enormous amounts of the drug to be absorbed (420 to 1880γ daily). Such huge doses of stilbestrol far in excess of physiologic requirements, caused toxic changes in about one-third of the rats. No evidence of the tumorigenic action of stilbestrol was found.

The authors wish to thank Burroughs Wellcome & Co. for material and aid furnished for this experiment.

REFERENCES

1. Russell, H. K., R. C. Page, C. S. Matthews, E. L. Schwabe and F. E. Emery: *Endocrinology.* 28: 897. 1941.
2. Emery, F. E., et al: *J. Clin. & Lab. Med.* In press.
3. Emery, F. E., L. M. Emery and L. Schwabe: *Growth* 4: 17. 1940.
4. MacBryde, C. M.: H. Freedman, E. Loeffel and D. Allen: *Proc. Soc. Exper. Biol. & Med.* 43: 212. 1940.
5. Von Haam, E., M. A. Hammel, I. E. Ravdin and R. H. Schoene: *Endocrinology* 28: 263. 1941.
6. Wolfe, J. M.: *Am. J. Physiol.* 115: 665. 1936.
7. Selye, H.: *J. Pharmacol. & Exper. Therap.* 71: 236. 1941.
8. Lipschutz, A., and L. Vargas, Jr.: *Lancet* 1: 541. 1940.
9. Dodds, E. C., W. Lawson and R. L. Noble: *Lancet* 1: 1389. 1938.
10. Sondern, C. W., and J. L. Sealey: *Endocrinology* 27: 670. 1940.
11. Dodds, E. C., L. Goldberg, W. Lawson and R. Robinson: *Nature* 141: 247. 1938.

INCIDENCE OF HOMOPLASTIC ADRENOCORTICAL TRANSPLANTS IN GONADECTOMIZED RATS

LELAND C. WYMAN AND CAROLINE TUM SUDEN

From the Physiological Laboratory of Boston University School of Medicine and the Evans Memorial, Massachusetts Memorial Hospitals

BOSTON, MASSACHUSETTS

THE 'end organ' hormone hypothesis, that the production of a stimulating hormone by the anterior pituitary gland is limited by a suppressing effect of an excess of the hormone of the gland which is stimulated, has been developed to explain the rhythmicity of the female sexual cycle, the hypertrophy of endocrine organs in states of deficiency, and the failure of grafts of certain endocrine tissues in animals possessing intact cognate glands (Halsted's 'law of deficiency'). Although applied most extensively to pituitary-gonadal relationships, the hypothesis has been applied to pituitary control of the thyroid and of the adrenal cortex (see reference 1, p. 496).[1]

Besides the direct approach to establishing the validity of this hypothesis, there is another, that removal of one 'end-organ' (e.g. the gonads) may 'release' the production of 'end-organ' stimulating hormones other than the one specific for the organ removed (e.g. corticotropin). That this may be profitable is shown by the agreement (in at least 11 recent independent reports) that castration of male mice or rats is followed by an increase in the weight of the adrenal glands. In female animals, however, ovariectomy is said to be followed by no change or a decrease in adrenal weight, the reports being about equally divided as to the two sequelae (2–7).

It has been established that the growth of transplanted adrenocortical tissue depends upon pituitary activity (presumably corticotropin production) (8). Intramuscular autoplastic transplants are successful in about 95% of cases (based on a study of 430 cases), but non-sibling homoplastic transplants are successful in about 25 or 30%. The effect of gonadectomy upon this latter incidence was therefore studied for comparison with the results on adrenal weights, and to further clarify the reported sex differences.

METHODS

An inbred strain of albino rats, reared on a diet of Purina Chow and greens, was used. Homoplastic transplants of adrenal cortex were made in the dorsal body muscles of young mature, adrenalectomized, non-sibling recipients. Gonadectomy was performed simultaneously, except for 3 series which were gonadectomized 2 weeks, 2 months, and 3 months previously (table 1). The controls were treated likewise but not gonadectomized. Successful transplantation was judged by health, activity, survival and gross and microscopic examination of the grafts at biopsy, 60 to 125 days after transplantation.

RESULTS

A gross summary of the results is presented in table 1. The incidence of successful grafts in the male non-gonadectomized controls was 32.1% and in the female controls

Received for publication April 15, 1941.

[1] Since the literature bearing on the problems discussed in this paper is so voluminous, only a few key references will be cited, the bibliographies of which may guide the interested reader. It is not the intention to slight the contributions of the many authors who have discussed the subject.

TABLE 1

Description	Age at transplantation, days	No. of rats	No. of successful transplants	%
Male Rats				
Non-castrated controls	77–110	28	9	32.1
Castrated at time of homotransplantation	77–111	39	21	53.8
Castrated 2 weeks before transplantation	88– 89	10	4	40.0
Castrated 2 and 3 months before transplantation	117–150	45	12	26.7
Female Rats				
Non-ovariectomized controls	71–184	64	16	25.0
Ovariectomized at time of transplantation	71–184	36	11	30.5

it was 25.0%. This differs from figures previously published by us (8, 9), being somewhat higher for males but remarkably lower for females. As yet we know of no difference in the treatment of the present and the previous series, except that the number in the previous series (10 males, 14 females) may have been too small for reliability. The increased incidence in the simultaneously gonadectomized male series (53.8%) is significant. The slightly higher incidence in the gonadectomized female series (30.5%) is not great enough to be proved statistically significant.

In the male series castrated 2 weeks before homotransplantation the incidence of successful grafts was 40.0%. This is above that of the control series, but not greatly so, and although the number, 10, was small, it may indicate that the influence which apparently increases the incidence when castration and transplantation are simultaneous was already disappearing. In the series of males castrated 2 or 3 months before transplantation the incidence was 26.7%, a figure not significantly different from that of the controls.

DISCUSSION

The older work on adrenal weight after gonadectomy in a variety of species is confusing and contradictory, and much of it may be criticized for various reasons (2). The majority of authors report an increase in both sexes, although more instances of no change or a decrease are listed for females. More recent work on mice and rats is accordant in reporting an increase in adrenal weight in castrated males and no change or a decrease in females (see above). The increased incidence of successful homoplastic adrenocortical transplants in castrated male rats and lack of significant change in females correspond with these latter reports.

These findings together with the reported decrease in adrenal weight following administration of testosterone (5, 7, 10), and the large body of evidence that androgens suppress the gonadotropic activity of the pituitary (increase in pituitary weight and gonadotropin content, and evidence of basophilic activity after castration, and the opposite effects after androgen administration), confirm the 'end-organ' hormone hypothesis for a pituitary-androgen relationship. They also confirm the extension of the hypothesis, namely the possibility that activity of one 'end-organ' may suppress activities of the pituitary other than the production (or release) of its own specific tropin. The situation regarding a pituitary-estrogen relationship in females, however, appears to be different.

In spite of the large amount of past and still accumulating evidence that estrogens suppress pituitary activity and that the 'end-organ' hormone hypothesis applies equally to both sexes, recent evidence is rapidly growing which indicates that estrogens also stimulate anterior lobe activity (11, p. 69). No change or a decrease in pitui-

tary weight after ovariectomy (2, 3, 4, 7, 12), increase in size and metabolic rate of the pituitary and cytological signs of greater secretory activity after administration of estrogens (3, 13–16), better survival of pituitary grafts under estrogen treatment (17), and other types of evidence support this view. With regard to corticotropic activity there are the larger adrenal glands of females, greater regeneration of cortical tissue in grafts in female rats (18), greater ability of female pituitary implants to cause adrenal hypertrophy (19), increase in adrenal weight after estrogen administration (3, 14), no change or a decrease in adrenal weight after ovariectomy, and the present findings that the incidence of successful homoplastic adrenocortical transplants is not significantly changed by ovariectomy. These sex differences could be explained if estrogens do stimulate corticotropic function, or at least if they do not exert an inhibitory effect similar to that proposed for androgens.

It is not the province of this paper to discuss the effects of this evidence upon the classical 'alternation' theories, or the possible reasons for the contradictions (e.g., dosage effects, chemical differences in supposedly similar hormones, etc.). Such a discussion may be found in reference 11, p. 100. It must be recognized, however, that an alternative hypothesis not involving the pituitary has been proposed to explain adrenal atrophy after ovariectomy, i.e., an increased rate of cell destruction in the cortex because of greater functional demand (6).

There is not complete agreement as to the duration of pituitary changes induced by gonadectomy. The increase in the gonadotropin content of the pituitary of female rats is said to reach a maximum in about 60 days and to remain at that level for at least 6 to 8 months (20–23), and the cytological changes are said to persist for years (16). According to our findings the changes responsible for the increased incidence of successful homoplastic adrenocortical grafts in male rats when castration and transplantation are simultaneous do not persist indefinitely, and may begin to wane within 2 or 3 weeks.

SUMMARY

When gonadectomy is performed simultaneously with homoplastic transplantation of adrenal cortex the incidence of successful grafts in male rats is greater than that in non-gonadectomized controls, but in female rats it is not significantly changed. In male rats castrated two or three months before transplantation the incidence of successful grafts is not significantly different from that of the controls. These results are discussed in terms of the effect of gonadectomy upon the anterior pituitary gland.

REFERENCES

1. Swann, H. G.: *Physiol. Rev.* 20: 493. 1940.
2. Andersen, D. H., and H. S. Kennedy: *J. Physiol.* 79: 1. 1933.
3. Lausen, H., C. G. Heller and E. L. Sevringhaus: *Endocrinology* 21: 735. 1937.
4. Chiodi, H.: *Rev. Soc. Argent. de Biol.* 14: 246. 1938.
5. Hall, K., and V. Korenchevsky: *J. Physiol.* 91: 365. 1938.
6. Blumenfeld, C. M.: *Endocrinology* 24: 723. 1939.
7. Mazer, M., and C. Mazer: *Endocrinology* 26: 662. 1940.
8. Wyman, L. C., and C. tum Suden: *Endocrinology* 21: 523. 1937.
9. Wyman, L. C., and C. tum Suden: *Science* 85: 589. 1937.
10. Mazer, M., and C. Mazer: *Endocrinology* 24: 175. 1939.
11. Evans: Recent Advances in Physiology, 6th ed. Blakiston, Philadelphia. 1939.
12. Brolin, S. E.: *Endokrinol.* 22: 331. 1940.
13. Weil, A., and B. Zondek: *Endocrinology* 25: 114. 1939.
14. Uotila, U. U.: *Endocrinology* 26: 123. 1940.
15. Victor, J., and D. H. Andersen: *Am. J. Physiol.* 120: 154. 1937.
16. Severinghaus, A. E.: *Physiol. Rev.* 17: 556. 1937.
17. Phelps, D., E. T. Ellison and J. C. Burch: *Endocrinology* 25: 227. 1939.
18. Wyman, L. C., and C. tum Suden: *Am. J. Physiol.* 101: 662. 1932.
19. Emery, F. E., and C. A. Winter: *Anat. Rec.* 60: 381. 1934.
20. Evans, H. E., and M. E. Simpson: *Am. J. Physiol.* 89: 371. 1929.
21. Korenchevsky, V., and M. Dennison: *J. Path. & Bact.* 38: 231. 1934.
22. Lausen, H. D., J. B. Golden and E. L. Sevringhaus: *Endocrinology* 25: 47. 1939.
23. Hellbaum, A. A., and R. O. Greep: *Am. J. Anat.* 67: 287. 1940.

THE EFFECT OF RENIN ON THE URIC ACID METABOLISM OF THE PREGNANT AND NON-PREGNANT DALMATIAN DOG[1]

N. K. SCHAFFER, L. V. DILL AND H. J. STANDER

From the Department of Obstetrics and Gynecology, Cornell University Medical College and the New York Hospital

NEW YORK CITY

IN CONSIDERING possible factors which may be involved in the hyperuricemia of eclampsia (1, 2), hypertension is suggested by reports (3) of a hyperuricemia in arterial hypertension without renal insufficiency. For this reason we have investigated the effect of single and repeated pressor doses of renin on the blood and urine uric acid of the pregnant and non-pregnant Dalmatian dog. In addition an experiment is included on the effect of experimental hypertension of renal ischemic origin on the blood uric acid of the pregnant Dalmatian dog.

EXPERIMENTAL

The Dalmatian dogs used in these experiments were normal pregnant and non-pregnant bitches and a male. They were high uric acid producers as determined by the criterion that the uric acid nitrogen : creatinine nitrogen ratio of a random sample of their urine was 0.5 or greater.[2]

Renin was prepared from pig kidney essentially by the method of Helmer and Page (4); 3000 cc. of 2% sodium chloride solution in 2 or 3 extractions, instead of 1000 cc., was used in extracting the kidney powder. Hyflo super-cel was employed in all the filtrations. The filtrate of the pH 4.5 precipitation was adjusted to pH 6.8 and concentrated in vacuo to about 800 cc. for the phosphate precipitation. Fractions *C* and *D* were used for intravenous injection. They were made to 50 cc. per kg. of fresh tissue and preserved with 0.02% merthiolate. Their nitrogen contents were 9.6 and 3.2 mg. per cc., respectively. They gave pressor responses with the amounts administered. For example, *dog A* weighing 25 kg. gave pressor responses of 40 and 50 mm. Hg with 0.5 cc. of fraction *C*.

Renal ischemia was produced by partial renal artery constriction[3] with a flank approach. The left renal artery was constricted with a silver clip to about two-thirds of its diameter so that there was slight if any pulsation distal to the clip. Three days later the right renal artery was constricted to about half of its diameter.

Blood uric acid was determined on whole blood by the micromethod of Folin (5). For accurate measurement at the small concentrations in Dalmatian dog's blood it was necessary to correct for the blank obtained with the reagents and to select a standard close to the unknown. In one experiment, the blood uric acid was determined by the indirect method of Benedict and Hitchcock (7). We deviated from this

Received for publication June 5, 1941.

[1] Supported by a grant from the John and Mary R. Markle Foundation.

[2] Trimble, H. C., personal communication.

[3] We are greatly indebted to Doctors George M. Knapp and Earl P. Lasher, Jr. of the Department of Experimental Surgery for this operation.

method in making the final dilution to 100 cc. instead of 50 cc. in order to obtain a more suitable range of color intensity for reading with the photoelectric colorimeter. The proportionality of the method was unaffected by this higher dilution. When protein was present in the urine,[4] the uric acid determinations were made on tungstic acid filtrates (8) of all the urines in the experiment. In one experiment urine uric acid was determined by the uricase method.[5] Blood urea was determined by the manometric hypobromite method of Van Slyke and Kugel (9). Urine urea after permutit treatment and urine urea plus ammonia were similarly determined (10). Blood sugar was determined by the macromethod of Benedict (11). Blood pressure was measured by direct femoral arterial puncture.

RESULTS

Effect of a single renin injection on blood and urine uric acid of the Dalmatian dog. The effect of a single dose of renin on the blood uric acid and uric acid excretion was

TABLE 1. EXPERIMENTS ON EFFECT OF A SINGLE RENIN INJECTION ON BLOOD AND URINE URIC ACID OF THE DALMATIAN DOG

No.	Dog	Renin Fraction	Dose	Rise in blood uric acid	Rise in uric acid excretion	
			cc.	%	%	
1	A	C	1.5	40		3 days antepartum
2	A	C	1.5	32		2 days antepartum
3	A	C	1.4	51	190	6 days postpartum
4	A	C	2.5	31	63	31 days postpartum
5	A	D	1.5	23	88	Middle of pregnancy
6	B	D	1.5	7	10	Middle of pregnancy
7	C	C	2.8	60		Male

studied in 7 experiments (table 1). Five experiments were carried out with *dog A*, 3 during pregnancy and 2 after it. A rise in blood uric acid occurred in each instance amounting to 23 to 51%. The uric acid excretion, examined in 3 of these experiments, was found to increase from 63 to 190%. A typical experiment (No. 4) is given in table 2. A steady increase in the uric acid excretion was obtained reaching a maximum, 63% above the initial rate, at about 4.5 hr. after the injection and remaining elevated for at least 4 hr. The blood uric acid rose 25% at 40 min. after the injection, then fell and rose 31% and did not approach the initial level until about 8 hr. after the injection. These changes were shown to be due to true uric acid by parallel determinations with the uricase method. From the experiments that were continued long enough it appears that the blood uric acid returns to normal before the uric acid excretion.

In most of these experiments the dog exhibited a variable reaction after the injection characterized by such behavior as barking, mild struggling, generalized vasodilatation, defecation, vomiting, lacrimation and salivation. This occurred with both renin fractions *C* and *D*.

A control experiment with the injection of 1.5 cc. of normal saline was made on *dog A* at 10 days postpartum. Over a period of 7 hr. the uric acid excretion was 35.8, 33.3, 35.8 and 38.5 mg. per hr. and the blood uric acid was 0.56, 0.51 and 0.57 mg.%. Thus both remained relatively constant. The blood uric acid was studied in another control experiment (on *dog A* at 52 days postpartum) using 2 cc. of fraction C that

[4] Protein was found in the urine after renin injections. Evidence that the dark pigment of fraction C is excreted is given by the darker color of the urine after an injection. At times protein was found in the urine after catheterization without a renin injection.

[5] The application of the uricase method to the determination of urine uric acid will be reported separately.

had been inactivated by heating to 60° C. for 10 minutes. The dog had no apparent reaction except for a little vomiting at 2 hr. after the injection. The blood uric acid before the injection was 0.61 mg.%. The values distributed between 1.25 and 6.25 hr. after the injection were 0.64, 0.52, 0.61 and 0.59 mg.%. Thus the blood uric acid was unaffected by the inactivated renin.

TABLE 2. EFFECT OF A SINGLE RENIN INJECTION ON BLOOD AND URINE URIC ACID OF THE DALMATIAN DOG

Blood sample	Urine collection	Urine uric acid		Blood uric acid	
		Benedict mg./hr.	Uricase mg./hr.	Folin mg.%	Uricase mg.%
9:12				0.49	0.33
10:29	9:21–10:31	31.2	29.2	0.49	0.35
	2.5 cc. fraction C injected at 10:30				
11:42	10:31–11:49	36.6	31.4	0.61	0.46
1:22	11:49– 1:41	44.0	39.5	0.51	0.34
3:51	1:41– 3:46	50.9	45.7	0.64	0.52
6:36	3:46– 6:49	48.3		0.54	0.37

Dog A at 31 days postpartum. Dog starved over night and water also withdrawn during experiment. Short reaction few min. after injection characterized by generalized vasodilatation, defecation, vomiting, lacrimation and salivation. Urine collected by catheter and bladder rinsed after each collection. Weight 20.5 kg.

The effect of a renin injection on the blood and urine urea as well as uric acid was studied in two experiments (table 3). In the first experiment using fraction C, it is seen that in the first 1.5 hr. after the injection the excretion of urea and non-protein nitrogen had decreased about 75%, when the uric acid excretion had increased 65%. Following this fall, the urea excretion rose above the control rate, but the average excretion was still 29% below it. The blood urea increased 19% above the first value obtained 13 min. after the injection, while the uric acid excretion and blood uric acid rose 190 and 51%, respectively.

In the second experiment, a more purified renin preparation, fraction *D*, was used. The results differed from those of the preceding experiment in that there was no initial decrease in the excretion of urea plus ammonia, but a steady increase reaching 83%. The blood urea rose 12%. The uric acid excretion rose 88%, while the

TABLE 3. EFFECT OF A SINGLE RENIN INJECTION ON BLOOD AND URINE URIC ACID AND UREA OF THE DALMATIAN DOG

Exp.	Blood sample	Urine collection	Urine uric acid mg./hr.	Urine urea N mg./hr.	Urine non-protein N mg./hr.	Blood uric acid mg.%	Blood urea N mg.%
1.		10:22–11:51	29.8	272	336		
		1.4 cc. fraction C injected at 11:46					
	11:59	11:51– 1:19	49.4	59	106	0.99	21.9
	12:50					1.37	23.7
	2:25	1:19– 2:49	86.4	233	317	1.49	26.1
	4:23	2:49– 4:26	69.5	290	371	1.40	25.2
2.	11:12	9:02–11:13	25.9	228[1]		0.31	14.2
		1.5 cc. fraction D injected at 11:13					
	12:39	11:13– 1:22	48.8	361		0.38	15.3
	3:11	urine lost				0.38	15.7
	5:05	3:42– 5:24	34.2	416		0.36	15.9
		5:24– 7:03	31.5	406			

[1] Urine urea+ammonia N was determined in this experiment.

Exp. 1 with *dog A* at 6 days postpartum. Short reaction of barking, mild struggling and defecation with vomiting and diarrhea later.

Exp. 2. with *dog A* at middle of 2nd pregnancy. Pressor response of 50 mm. Hg obtained. Mild reaction of salivation, generalized vasodilatation and defecation. Blood sugar results in mg.%: control 90, after injection 108, 97 and 94. Weight 20.5 kg.

blood uric acid rose 23%. The blood sugar rose 17%. This experiment suggests that renin stimulates the excretion of urea as well as uric acid and that probably if the preceding experiment had been carried on longer a similar increase would have been found. Moreover, together with the evidence in the following experiment, it indicates that renin has a hyperglycemic action.

The response of another Dalmation bitch *B* of 15.8 kg. body weight was studied in the middle of pregnancy with 1.5 cc. of fraction *D*. No reaction was noticeable. The uric acid excretion and blood uric acid increased only 10 and 7%, respectively. This small effect on uric acid metabolism may be associated with an unusually high control uric acid excretion of 47.3 mg. per hr. or may be due to individual variation.

TABLE 4. EFFECT OF REPEATED RENIN INJECTIONS ON BLOOD AND URINE URIC ACID OF THE DALMATIAN DOG

Antepartum day	Urine collection *hr.*	Urine uric acid *mg./hr.*	Blood uric acid *mg.%*	Doses of fraction C *cc.*
6th	26.0	21.8		Control day
5th	28.2	23.7	0.42	1.5, 1, 1
5th	16.2	24.8	0.51	0.5, 0.5, 0.5, 0.5
3rd	20.3	30.4	0.43	0.5, 1.5
2nd	23.7	42.9	0.34	0.5, 1.5

Dog A in last week of pregnancy. Injected at 4 to 12 hr. intervals. Condition of dog remained good. Blood samples were taken at beginning of day before an injection. Urine was collected in metabolism cage and dog was catheterized for rinsing of bladder at beginning and end of day. Non-protein N excretion on 3rd and 2nd antepartum days was 238 and 296 mg./hr.; blood non-protein N was 32.1 and 33.8 mg.% on same days. Diet consisted of dog biscuits fed ad libitum. Weight 25 kg.

The excretion of urea plus ammonia rose 20%. The blood sugar rose 16%, from 92 to 109 mg.% at 52 min. after the injection.

A male Dalmatian *C* of 25.7 kg. body weight was given 2.8 cc. of fraction *C*. A mild reaction followed with defecation and vomiting. The blood uric acid increased from 0.55 mg.% before the injection to 0.88 mg.% at 4 hr. after injection (60% rise).

A non-pregnant mongrel bitch of 10.5 kg. body weight was injected with 2.5 cc. of fraction *C*. There was a delayed reaction of defecation, vomiting and vaginal mucous discharge. The blood uric acid rose from 0.19 mg.% before the injection to 0.30 mg.% at 4 hr. after it (58% rise). This experiment suggests that renin produces a similar effect in mongrel dogs.

Effect of repeated renin injections on blood and urine uric acid of the Dalmatian dog. Dalmatian dog *A* was given repeated doses of fraction *C* 2 to 4 times a day at 4 to 12 hr. intervals over a period of 4 days in the last week of pregnancy (table 4). The uric acid excretion increased continuously during the injection period and on the last day was 97% above the control value. The blood uric acid increased temporarily after the injections, but the level examined at the beginning of each injection day did not seem to change significantly, except for a possible small downward trend in the last 3 days paralleling the upward trend of uric acid excretion.

Effect of experimental hypertension on the blood uric acid of the pregnant Dalmatian dog. Hypertension was produced in the Dalmatian bitch *A* in the second pregnancy. The left renal artery was constricted at 11 days antepartum. Three days later the right renal artery was constricted. The effect on blood uric acid, non-protein nitrogen, sugar and blood pressure is given in table 5. The ratio, 1000 × uric acid : nonprotein nitrogen, is also given, since it minimizes uric acid rises that accompany non-protein nitrogen rises. On the second day after the first constriction the uric acid rose, but it was associated with a postoperative rise in non-protein nitrogen. On the third day the uric acid rose 35% and the ratio rose 41% above the values before the operation,

TABLE 5. EFFECT OF EXPERIMENTAL HYPERTENSION ON THE BLOOD URIC ACID OF THE PREGNANT DALMATIAN DOG

Date	Nov. 29	Dec. 10	11	12	13	14	15	16	17	18	19	20	21	22	23	28	Jan. 1
Blood uric acid, mg.% A	0.33	0.34	0.37	0.47	0.46	0.36	0.62	0.49	0.51	0.52	0.65	0.74	0.51	0.36	0.58	0.49	0.42
Blood non-protein N, mg.% B	35.0	38.2	46.9	58.0	36.8	37.3	46.2	49.4	35.8	39.4	36.1	57.4	41.1	41.3	51.1	40.7	44.9
1000 A/B	9.4	8.9	7.9	8.1	12.5	9.7	13.4	9.9	14.3	13.2	18.5	12.9	12.4	8.7	11.4	12.0	9.4
Blood sugar, mg.%	90	95						134	112		116	112		88			
Blood pressure, mm. Hg	140	110	165	130	140	125	150	150	165	175	155		170		150	145	130

Dog A in last 2 weeks of 2nd pregnancy. Dec. 10, left renal artery constricted after blood sample. Dec. 13, right renal artery constricted after blood sample. Dec. 20, normal delivery of 5 puppies before blood sample. Dec. 23, ate 1 puppy before blood sample. Clinical condition of dog remained good. Albuminuria in gm. per liter: Dec. 10–17 up to 0.1, Dec. 18–19, 0.2–0.25, Dec. 20–Jan. 1 up to 0.05. Diet of canned dog food kept constant. Weight 25 kg. on Dec. 10.

while the non-protein nitrogen was normal. On the day after the second constriction the uric acid and blood pressure fell to normal. From the following day until delivery the uric acid and blood pressure rose and remained elevated, while the non-protein nitrogen rose for 2 days and fell to normal during the last 3 days before delivery. The increase in uric acid and ratio in this period reached as high as 81 and 91% and averaged 56 and 58%, respectively, above the normal values on the day after the second constriction. The greatest uric acid rise occurred on the delivery day, but this was accompanied by a non-protein nitrogen rise. The uric acid and ratio were normal on the second day postpartum, but were increased on the next day because the dog ate a puppy. On the twelfth day postpartum all values were approaching normal. A sustained hyperglycemia also occurred in this experiment, amounting to 41% maximally and averaging 27%.

DISCUSSION

The stimulating effect of renin on uric acid excretion would seem to be explained partially at least by the fact that renin produces diuresis (12) and diuresis is known to increase uric acid excretion (13). However, the increase in blood uric acid would not be expected to arise from diuresis (13), therefore some other factor is indicated. The uric acid changes may have been influenced by the mild reactions accompanying the injections, but in one experiment (No. 2) a 32% rise in blood uric acid occurred without a noticeable reaction.

The hyperglycemic effect of renin confirms the similar finding of Foglia and Moglia (14) with hypertensine (angiotonin) in dogs. The effect of renin on uric acid metabolism and blood sugar is somewhat like that of non-pressor doses of adrenaline (15). It is interesting in this connection that the hyperglycemic action of hypertensine is abolished by adrenalectomy (14).

Single injections of renin produced rises of 19 and 12% in the blood urea. The effect on urea excretion was variable. In one case an average decrease of 29% occurred, whereas in two other cases it rose maximally 83 and 20%. Renin previously has been found to increase the urea excretion in rabbits and this has been interpreted on the basis of its diuretic action (12). As pointed out above, however, a rise in blood uric acid and urea accompanying an increased excretion of these substances would indicate some factor in addition to diuresis, for example, possibly an increase in catabolism.

The elevated blood uric acid and sugar obtained in the hypertensive pregnant Dalmatian dog are possibly related to the similar findings with single renin injections, since there is evidence that the hypertension is mediated by the renin-angiotonin vasopressor system (16). Although these results are consistent with the suggestion of the possible involvement of hypertension in the hyperuricemia of eclampsia, they merely offer a lead for further investigation.

SUMMARY

Single injections of renin in the pregnant and non-pregnant Dalmatian dog raised the uric acid excretion 31 to 190% and the blood uric acid 23 to 60%. Repeated injections over a period of 4 days produced a continuous rise in the daily uric acid excretion, reaching 97% above the initial excretion, whereas the blood uric acid was only temporarily raised after each injection. Experimental hypertension in a pregnant Dalmatian dog produced a sustained elevation in the blood uric acid level to as high as 81% and averaging 56% without affecting the blood non-protein nitrogen except postoperatively.

The blood sugar rose 17% after single renin injections. In the hypertensive preg-

nant dog a sustained hyperglycemia was obtained with a maximal rise of 41% and an average rise of 27%.

REFERENCES

1. Cadden, J. F., and H. J. Stander: *Am. J. Obst. & Gynec.* 37: 37. 1939.
2. Schaffer, N. K., and H. J. Stander: *Proc. Soc. Exper. Biol. & Med.* 45: 180. 1940.
3. Brøchner-Mortensen, K.: *Medicine* 19: 208. 1940.
4. Helmer, O. M., and I. H. Page: *J. Biol. Chem.* 127: 757. 1939.
5. Folin, O.: *J. Biol. Chem.* 101: 111. 1933; 106: 311. 1934.
6. Blauch, M. B., and F. C. Koch: *J. Biol. Chem.* 130: 443. 1939.
7. Benedict, S. R., and E. H. Hitchcock: *J. Biol. Chem.* 20: 619. 1915.
8. Peters, J. P., and D. D. Van Slyke: Quantitative Clinical Chemistry. Methods. Williams & Wilkins, Baltimore, p. 535. 1932.
9. Van Slyke, D. D., and V. H. Kugel: *J. Biol. Chem.* 102: 489. 1933.
10. Van Slyke, D. D., I. H. Page, A. Hiller and E. Kirk: *J. Clin. Invest.* 14: 901. 1935.
11. Benedict, S. R.: *J. Biol. Chem.* 92: 141. 1931.
12. Pickering, G. W., and M. Prinzmetal: *J. Physiol.* 98: 314. 1940.
13. Brøchner-Mortensen, K.: *Acta Med. Skand. Suppl.* 84: 132. 1937.
14. Foglia, V. G., and J. L. Moglia: *Rev. soc. argent. de biol.* 16: 529. 1940.
15. Chaikoff, I. L., P. S. Larson and L. S. Read: *J. Biol. Chem.* 109: 395. 1935.
16. Page, I. H.: *J. Exper. Med.* 72: 301. 1940.

INFLUENCE OF HORMONES UPON PHOSPHATASE CONTENT OF RAT FEMURS[1]

I. EFFECTS OF ADRENAL CORTICAL SUBSTANCES AND PARATHYROID EXTRACT

H. L. WILLIAMS AND E. M. WATSON
From the Department of Pathological Chemistry
University of Western Ontario
LONDON, CANADA

AS AN ATTEMPT to explain the apparent salutary effects of adrenal cortical extract when administered therapeutically to persons with Paget's disease of the bone (1, 2, 3) and chronic arthritis (4), the experiments described below were undertaken.

In addition to more or less pronounced clinical improvement, the treatment of patients with these diseases by means of adrenal cortical extract has been found to be accompanied by a reduction of the serum phosphatase activity. The latter effect might be explainable in several ways, e.g., inactivation of the catalytic properties of the enzyme in the organism as a result of the treatment, increased elimination of the phosphatase by way of the intestine, biliary tract or kidney, or decreased rate of its production or delivery to the blood stream.

We have performed experiments which show that adrenal cortical extract does not modify the activity of bone phosphatase in vitro. No data are at hand which demonstrate an excessive excretion of the phosphatase from the circulating fluids of persons with Paget's disease as a result of therapy. The third possibility, namely, a modification of the production of the enzyme in the bones is the subject of the present investigation.

Some evidence has been adduced that the adrenal glands may be concerned, directly or indirectly, with osseous metabolism. For example, Rogoff and Stewart (5) observed hypercalcemia in adrenalectomized animals and Rogoff (6) postulated a functional interrelationship between the adrenal and parathyroid glands. Schour and Rogoff (7) demonstrated that adrenalectomy caused abnormalities of the teeth of rats similar to those produced by the injection of parathyroid extract. Pugsley and Collip (8) found that adrenalectomized rats, maintained on a 1% solution of sodium chloride, showed a greater increase of the serum calcium and the excretion of calcium after the injection of parathyroid extract than did control animals. Berman (1) intimated that Paget's disease might be related to an imbalance between the parathyroid and adrenal glands.

The phosphatase of the bones in experimental hyperparathyroidism has been studied by others with variable results. Page (9) reported that parathormone decreased the phosphatase activity of the bones while Roche and Filippi (10) noted that small doses of the material produced a marked rise in the phosphatase activity

Received for publication May 15, 1941.
[1] These investigations were supported by a grant from the Associate Committee on Medical Research of the National Research Council, Ottawa, Canada.

which persisted for at least 12 days. As indicated by the findings described below, the apparent divergence of these results may be accounted for on the basis of differences of the duration of the treatment as well as differences of the dosages employed. Heretofore, not any observations have been presented, apparently, with respect to the influence of adrenal cortical preparations on the phosphatase content of bones.

METHODS

The method of estimation of the relative concentration of the phosphatase in the bones (11) consisted essentially of extracting the enzyme from the femurs (the diaphyses and epiphyses being analyzed separately) by means of the procedure described by Martland and Robison (12). After two days' autolysis, the extracts were filtered, dialyzed well and diluted to a definite volume. The dry, fat-free weight of the residual bony fragments was determined after extraction with alkaline ether in a micro-extractor (Pickel). The measure of the reaction velocity was obtained as directed by O. Bodansky (13) under optimal conditions of pH, α-amino acid and magnesium ion concentrations and at a constant temperature of 25° C. All phosphate estimations were made according to the modified Kuttner-Lichtenstein (14) procedure with the employment of a Cenco-Sheard-Sanford photelometer.

The measure of the reaction velocity thus attained was divided by the dry, fat-free weight of the bones to obtain the measure of the reaction velocity per gram of dry, fat-free bone. This value was calculated as a percentage of a comparable value derived from the control animals. Thus the final numerical result represents the phosphatase content of the bones of the treated rats as a percentage of the phosphatase content of the appropriate untreated control animals.

The accuracy of the method is such that when corresponding leg bones of a rat were extracted separately and the phosphatase activities ($Q_{0.05}$) (13) of the extracts estimated individually, the results agreed within about 2%. Between different animals the agreement was less exact but with litter mates of the same sex and weight, the difference was about 5%. The bone phosphatase of adult rats which were not litter mates varied not more than 10%.

In the chronic experiments referred to below, adult male rats were used, 2 of which were killed at the end of each specified time interval. In the critical experiments, only litter mates of the same sex were employed. For each experiment, the rats were divided into 2 groups, one group constituting the control animals. The final results, as given, represent the findings obtained in several typical experiments. The results have been averaged when comparable and independent of differences due to age or sex of the animals.

DATA

Chronic Experiments

Effects of repeated small doses of adrenal cortical and parathyroid extracts. The effects of small doses of these preparations administered daily over a period of 14 weeks upon the phosphatase content of the bones were observed.

Four groups of male rats of as nearly as possible the same age and weight were selected. One group served as untreated controls. Another group received 0.2 cc. of adrenal cortical extract;[2] another was given 0.1 cc. (2 U) of parathyroid extract (parathormone)[3] while the fourth group got both products in the above-mentioned dosages, the two extracts being administered separately and at different times of the day. All the injections were subcutaneous. At definite times after the beginning of

[2] Adrenal cortex extract (Upjohn).
[3] Furnished by Eli Lilly & Co.

these treatments, 2 rats from each group were killed and the bones dealt with in the manner described above. In all, 40 animals were used in this series of experiments.

It was found that the adrenal cortical extract and parathyroid extract affected the phosphatase of the diaphyses and the epiphyses differently. The results are depicted in figure 1 and summarized below.

(1) *Diaphyses.* (*a*) *Adrenal cortical extract.* Under the influence of 0.2 cc. of adrenal cortical extract per day, the alkaline phosphatase of the diaphyses remained normal within reasonable limits. The tendency seemed to be, however, for the values to be somewhat low for the first part of the experiment and somewhat high for the remainder. Nevertheless, the small order of the changes are probably insignificant (fig. 1, curve A).

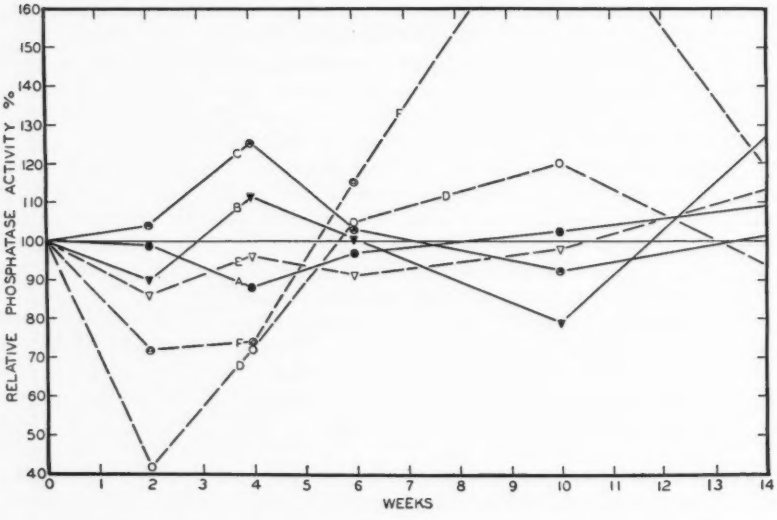


Fig. 1. Effects of repeated, small, injected doses of adrenal cortical and parathyroid extracts on the phosphatase content of the femurs of rats. *Diaphyses*: A, 0.2 cc. cortical extract, B, 0.1 cc. parathyroid extract daily, C, cortical extract plus parathyroid extract. *Epiphyses*: D, 0.2 cc. cortical extract, E, 0.1 cc. parathyroid extract daily; F, cortical extract plus parathyroid extract.

(*b*) *Parathyroid extract.* With the injection of 0.1 cc. (2 U) of parathormone, more pronounced variations occurred. During the first 2 weeks of treatment, the phosphatase declined, then rose above normal during the succeeding 2 weeks. A second more marked fall was registered followed by a pronounced rise (fig. 1, curve B).

(*c*) *Cortical extract plus parathyroid extract.* The combined effect of adrenal cortical extract and parathormone when given concurrently in the doses mentioned above was of the same general order as that produced by parathormone alone. Obviously, the cortical extract did not inhibit the tendency of the parathormone to increase the phosphatase of the diaphyses (fig. 1, curve C).

(2) *Epiphyses.* (*a*) *Adrenal cortical extract.* The epiphyses of the rats which received the cortical extract exhibited a prompt decrease of their phosphatase content which reached a minimum at the end of 2 weeks. There followed an equally abrupt increase to a level in excess of the normal (fig. 1, curve D).

(*b*) *Parathyroid extract.* The results for the parathormone-treated animals fol-

lowed somewhat the same course as that shown for the shafts of the bones but at a consistently subnormal level (fig. 1, curve E).

(c) *Cortical extract plus parathyroid extract.* The parathormone when administered along with cortical extract did not modify, apparently, the action of the latter (fig. 1, curve F).

The adrenal cortical extract in contrast with the parathyroid extract exerted its most marked effect upon the phosphatase content of the epiphyses. Not any close relationship between these two substances was apparent with respect to the phosphatase mechanism of bone metabolism.

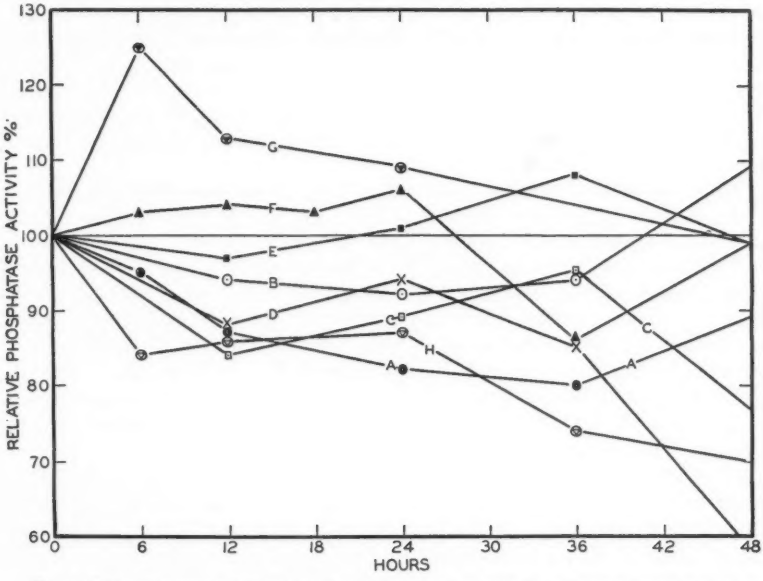


Fig. 2. Effects of single injections of adrenal cortical extract and related substances and parathyroid extract on the phosphatase content of the diaphyses of the femurs of rats. A, 5.0 cc. cortical extract (Upjohn, alcohol-free); B, 2.5 cc. cortical extract (Swingle); C, 2.0 mg. corticosterone; D, 2.0 mg. compound E; E, 5.0 cc. amorphous residue; F, 5.0 mg. desoxycorticosterone acetate; G, 1.0 cc. parathyroid extract; H, 3.0 cc. parathyroid extract.

Acute Experiments

Effects of single, large doses of adrenal cortical extract and related substances and of parathyroid extract. In this series of experiments the effects of individual, non-lethal doses of the various preparations were observed. Litter mates of the same sex were used; the ages of the litters ranged from 38 to 174 days but mostly less than 100 days. The animals of each litter were divided into 2 groups, one of which served as controls and received an amount of the solvent equivalent to that contained in the hormonal preparations. The results represent the findings in several such groups of animals.

(1) *Diaphyses.* (a) *Adrenal cortical extract.* After the injection of 3 cc. of cortical extract intraperitoneally, the phosphatase of the shafts of the femurs decreased markedly, the lowest value being noted at the end of 12 hours. Subsequently, it returned to a moderately low level which was maintained for at least 48 hours. The

injection of an amount of alcoholic saline equivalent to that present in 3 cc. of the extract showed that the first marked reduction of the phosphatase was due, in part at least, to this constitution of the preparation.

The administration of 5 cc. of the cortical extract from which the alcohol had been removed by distillation in vacuo below 45° C. caused a more prolonged diminution of the phosphatase, still evident 60 hours after the injection (fig. 2, curve A).

Evidence that this decreased concentration of the enzyme was not due to a direct "inactivating" effect of the extract upon the phosphatase in the bones was obtained by experiments in vitro. For example, under optimal conditions, the measure of the reaction velocity of the hydrolysis of the substrate was not altered when adrenal cortical extract was present in concentrations of 1:8 to 1:800. An extract of calf bone was used as the source of the phosphatase and control hydrolyses contained amounts of alcoholic saline comparable to that in the cortical extract.

The administration of 2.5 cc. of Swingle's adrenal cortical extract[4] produced a slight decrease of the phosphatase content of the diaphyses, but 48 hours after the injection a slight elevation was noted. The changes, however, were of doubtful significance (fig. 2, curve B).

(*b*) *Corticosterone.* After the injection of 2 mg. of corticosterone (Kendall's compound B),[5] the phosphatase content of the diaphyses decreased and so remained throughout the 48 hours of the experiment (fig. 2, curve C).

(*c*) *Compound E.* Similar results were obtained with 17-hydroxy, 11-dehydrocorticosterone (Kendall's compound E)[5] also in a 2 mg. dose (fig. 2, curve D).

(*d*) *Amorphous residue.* With the injection of 5 cc. of an amorphous residue[5] (1 cc. of which is capable of maintaining a 20 kg. adrenalectomized dog for one day) the diaphyseal phosphatase was unaffected (fig. 2, curve E).

(*e*) *Desoxycorticosterone acetate.* The administration of 5 mg. of desoxycorticosterone acetate in oil[6] tended to raise the phosphatase content of the diaphyses, although low values were obtained invariably after 36 hours (fig. 2, curve F).

(*f*) *Parathyroid extract.* Following the injection of 1 cc. (20 U) of parathormone, the phosphatase of the diaphyses rose to a maximum in about 6 hours and returned gradually to normal after 48 hours (fig. 2, curve G). With 3 cc. (60 U) a slight increase was noted followed by a rapid and continuous decrease (fig. 2, curve H).

Assuming that phosphatase is a product of the osteoblasts (15), these trends are understandable on the basis of Selye's studies of the histological changes in the bones following the injection of single large doses of parathyroid extract. He (16) demonstrated a proliferation of osteoblasts in the femur 6 hours after such injection. Subsequently, the osteoblasts disappeared, being replaced by osteoclasts so that 20 hours after the injection the picture of osteitis fibrosa existed.

(2) *Epiphyses.* (*a*) *Adrenal cortical extract.* The phosphatase content of the epiphyses decreased after the injection of 3 cc. of adrenal cortical extract to reach a minimum in about 6 hours with a return to normal in 24 hours. A second marked reduction was obvious at the end of 48 hours.

The first decrease, as before, was related apparently to the alcoholic content of the extract. The second more prolonged decrease was due to the adrenal substances as indicated in figure 3, curve A, which represents the results obtained with 5 cc. of the material from which the alcohol had been removed.

A marked but transitory reduction of the phosphatase was accomplished with 2.5 cc. of Swingle's cortical extract which was alcohol-free (fig. 3, curve B).

[4] Permission of Professor W. W. Swingle.
[5] Kindly supplied by Dr. E. C. Kendall of the Mayo Clinic.
[6] Percorten provided by the Ciba Company, Montreal.

(*b*) *Corticosterone.* Following the administration of 2 mg. of corticosterone, the epiphyseal phosphatase content decreased, with lowest values in 12 and 24 hours and a return to above the usual normal limits in 48 hours (fig. 3, curve C).

(*c*) *Compound E.* The injection of 2 mg. of 17-hydroxy, 11-dehydrocorticosterone yielded somewhat lower values than those referred to above with respect to corticosterone and which persisted for 48 hours (fig. 3, curve D).

(*d*) *Amorphous residue.* The injection of 5 cc. of a solution of the amorphous residue produced no regular or marked change in the epiphyseal phosphatase (fig. 3, curve E).

(*e*) *Desoxycorticosterone acetate.* As with the diaphyses, the administration of 5

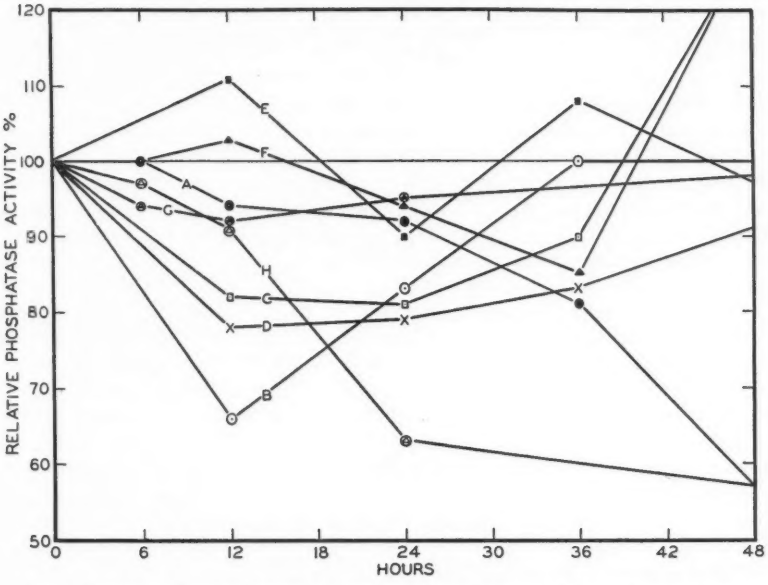


Fig. 3. EFFECTS OF SINGLE INJECTIONS OF ADRENAL CORTICAL EXTRACT AND RELATED SUBSTANCES AND PARATHYROID EXTRACT ON THE PHOSPHATASE CONTENT OF THE EPIPHYSES OF THE FEMURS OF RATS. A, 5.0 cc. cortical extract (Upjohn, alcohol-free); B, 2.5 cc. cortical extract (Swingle); C, 2.0 mg. corticosterone; D, 2.0 mg. compound E; E, 5.0 cc. amorphous residue; F, 5.0 mg. desoxycorticosterone acetate; G, 1.0 cc. parathyroid extract; H, 3.0 cc. parathyroid extract.

mg. of desoxycorticosterone acetate tended to increase the phosphatase of the epiphyses, although low values were obtained at 36 hours (fig. 3, curve F).

(*f*) *Parathyroid extract.* After the injection of 1 cc. (20 U) of parathormone, the phosphatase content of the epiphyses fell slightly during the first 24 hours and returned near to the normal level in 24 hours (fig. 3, curve G). With the 3 cc. (60 U) dose, the decrease was more pronounced and was still evident 48 hours after the injection (fig. 3, curve H).

It may be mentioned that the injection of from 5.0 to 7.5 mg. of dihydrotachysterol (A.T. 10),[7] which may be regarded as the synthetic counterpart of parathormone, caused a reduction of the phosphatase of both the diaphyses and epiphyses.

We found, also, that the injection of 1.0 mg. of calciferol[7] was followed by a pro-

[7] Obtained from the Winthrop Chemical Co. through the courtesy of Dr. H. A. Cave.

longed decrease of the phosphatase content of the diaphyses and the epiphyses. These findings may be related to osteoblastic degeneration such as Selye (16) observed after the administration of irradiated ergosterol.

DISCUSSION

While there is some indication that the adrenal cortical substances and parathormone influence the phosphatase content of the bones differently, there would appear to be no functional relationship between these hormones in this regard beyond that implied in the statment that, under certain specified conditions, they cause opposite effects but, under certain other conditions, they produce similar changes.

While a direct comparison between the results of experiments on rats and pathological conditions in human beings is not justifiable, a possible explanation is provided for the reduction of the serum phosphatase of patients with Paget's disease following treatment with adrenal cortical extract and the observed absence of this effect with the use of desoxycorticosterone acetate (3). Since the hyperphosphatasemia of Paget's disease is due to an actual increase of the enzyme in the blood (17) the result presumably of its passage in excessive amount from the affected bones and since there is apparently no direct deactivating effect of the extract, there remains but an action on the phosphatase within the bones. The results of the experiments reported herein tend to establish this thesis but it is not determined whether the hormonal substances act directly upon the skeletal tissues or indirectly through the intermediation of some metabolic process or endocrine function.

Numerous compounds have been isolated from the adrenal cortex which appear to exhibit more or less specific physiological effects. But, as mentioned by Kendall (18), no one compound can produce all of the known effects of the extract. Of the two cortical extracts investigated, that prepared according to the method of Cartland and Kuizenga[8] (19) was more effective in lowering the phosphatase content of bone than that of Swingle (20). This property of the former preparation was most nearly approximated by corticosterone and compound *E* (17-hydroxy, 11-dehydrocorticosterone) which are crystalline compounds with an oxygen attached at the 11th carbon atom of the steroid ring system. The lack of effect with the amorphous residue of Kendall and an opposite effect in the case of desoxycorticosterone acetate suggest a relationship between the action on the phosphatase and the chemical structure of the substances concerned, comparable to other qualitative differences which have been observed (21, 22) between the function and structure of the compounds derived from the adrenal cortex.

A certain parallelism exists between our findings and those of Wells and Kendall (22) who reported that those compounds obtained from the adrenal cortex with an oxygen on C_{11} when administered to adrenalectomized young rats retarded or actually suppressed the growth of the animals, whereas the amorphous fraction brought about an almost normal rate of growth. Desoxycorticosterone acetate, on the other hand, caused a gain in weight which was greater than normal. It has been shown (23) that an increase of phosphatase is associated with an increased rate of growth of the bones.

It is of interest to note that those compounds which were most effective in lowering the bone phosphatase were most active also (24), in their effect upon carbohydrate metabolism. Moehlig (25) has stressed a possible association between disturbed carbohydrate metabolism and Paget's disease.

---

[8] Adrenal cortex extract (Upjohn).

SUMMARY

The effects of parathyroid extract, adrenal cortical extract and related compounds upon the phosphatase content of the diaphyses and the epiphyses of rats' femurs have been observed.

Small repeated doses of parathyroid extract produced a temporary increase of the phosphatase of the diaphyses without alteration or that of the epiphyses. Large single doses of this product caused an increase, followed by a decrease, of the phosphatase of the diaphyses while only a decrease in the epiphyses was noted.

The injection of adrenal cortical extract caused a reduction of the phosphatase of the diaphyses and the epiphyses, the effect being more marked in the latter.

Of the adrenal cortical substances, corticosterone and the compound *E* of Kendall approximated most closely the effect of the whole cortical extract with respect to a reduction of the phosphatase content of the bones. The amorphous fraction of Kendall produced no regular or marked change of the bone phosphatase.

Desoxycorticosterone acetate caused an increase of the phosphatase content of the bones.

While the mechanism underlying these changes is not explained, a possible correlation may exist between the findings and the results of the clinical therapeutic use of adrenal cortical extract in Paget's disease of bone.

REFERENCES

1. Berman, L.: *Endocrinology* 16: 109. 1932.
2. Berman, L.: *Endocrinology* 20: 226. 1936.
3. Watson, E. M.: *Canad. M. A. J.* 41: 561. 1939.
4. Watson, E. M.: *Endocrinology* 27: 521. 1940.
5. Rogoff, J. M., and G. N. Stewart: *Am. J. Physiol.* 86: 25. 1928.
6. Rogoff, J. M.: *Science* 80: 319. 1934.
7. Schour, I., and J. M. Rogoff: *Am. J. Physiol.* 115: 334. 1936.
8. Pugsley, L. I., and J. B. Collip: *Biochem. J.* 30: 1274. 1936.
9. Page, I. H.: *Biochem. Ztschr.* 223: 222. 1930.
10. Roche, J., and A. Filippi: *Compt. Rend. Soc. de Biol.* 129: 326. 1938.
11. Williams, H. L.: *Univ. West. Ont. M. J.* 10: 101. 1940.
12. Martland, M., and R. Robison: *Biochem. J.* 23: 237. 1929.
13. Bodansky, O.: *J. Biol. Chem.* 114: 273. 1936, 118: 341. 1937; 120: 555. 1937; 129: 197. 1939.
14. Bodansky, A.: *Am. J. Clin. Path. Tech. Supp.* 1, 7: 51, 1937.
15. Franseen, C. C., and R. McLean: *Am. J. Cancer* 24: 299. 1935.
16. Selye, H.: *Endocrinology* 16: 547. 1932.
17. Williams, H. L., and E. M. Watson: *J. Lab. & Clin. Med.*, 26: 1333. 1941.
18. Kendall, E. C.: *J. Biol. Chem.* 133: li. 1940.
19. Cartland, G. F., and M. H. Kuizenga: *J. Biol. Chem.* 116: 57. 1936.
20. Swingle, W. W., and J. J. Pfiffner: *Am. J. Physiol.* 96: 153, 164, 180. 1931; 98: 144. 1931.
21. Kendall, E. C.: *Proc. Staff Meet. Mayo Clin.* 15: 297. 1940.
22. Wells, B. B., and E. C. Kendall: *Proc. Staff Meet. Mayo Clin.* 15: 133, 324. 1940.
23. Roche, J., A. Filippi and A. Leandri: *Bull. Soc. Chim. Biol.* 19: 1314. 1937.
24. Wells, B. B.: *Proc. Staff Meet. Mayo Clin.* 15: 294. 1940.
25. Moehlig, R. C.: *Surg., Gynec. & Obst.* 62: 815. 1936; 64: 747. 1937.

INFLUENCE OF HORMONES UPON PHOSPHATASE CONTENT OF RAT FEMURS[1]

II. EFFECTS OF SEX HORMONES, THYROXIN AND THYMUS EXTRACT

H. L. WILLIAMS AND E. M. WATSON

From the Department of Pathological Chemistry, University of Western Ontario Medical School

LONDON, CANADA

THE SECRETIONS of the gonads, the thyroid and thymus glands are important factors in the control of the development and growth of the organism including the skeletal system. Phosphatase is an accessory in the formation of osseous substance. The experiments described below were carried out to determine if the hormones influenced in any way the phosphatase content of the bones.

The methods of experimentation were essentially the same as those described in the foregoing paper, namely, the administration of single, large, non-lethal doses of the products under investigation to albino rats of approximately 50 days of age, killing the animals at intervals thereafter and estimating the relative phosphatase content of the femurs, the diaphyses and epiphyses being analyzed separately.

DATA

Diaphyses. (*a*) *Progesterone.* The injection of 5.0 mg. of progesterone,[2] in oil, caused a moderate increase of the phosphatase content of the diaphyses with a return to normal in 36 hours (fig. 1, curve A). Male and female rats, 49 days old, were used for this experiment.

(*b*) *Testosterone.* Five mg. of testosterone,[3] in oil, occasioned a somewhat greater increase of the diaphyseal phosphatase with its return to normal in 48 hours (fig. 1, curve B). In this experiment 58-day-old male rats were employed.

(*c*) *Estradiol.* Estradiol[3] (0.06 mg. and 1.5 mg. in oil) caused the phosphatase content of the diaphyses to decline slightly, reaching a minimum 6 hours after the injection. Thereafter, an irregular increase occurred which was still evident after 48 hours. The type and magnitude of the values seemed to be independent of the dosage employed (fig. 1, curve C). Females, 52 days of age, were used for the smaller dose of the hormone and 59-day-old females for the larger amount.

(*d*) *Thyroxin.* Thyroxin-sodium,[4] injected as a single dose of 1 mg., caused the diaphyseal phosphatase content to increase. Males 55 days of age yielded results similar to those obtained with 48-day-old females. The results are combined in figure 1, curve D.

(*e*) *Thymus extract.* Thymus extract[5] (2.0 cc.) caused the phosphatase content

[1] This work was supported by a grant from the Associate Committee on Medical Research of the National Research Council, Ottawa, Canada.

[2] Progestin, supplied by the British Drug Houses (Canada), Toronto.

[3] Supplied by the Ciba Company, Montreal.

[4] Supplied by Roche-Organon (Canada), Montreal.

[5] Supplied by Dr. L. G. Rowntree, Philadelphia.

of the diaphyses to decrease slightly 6 hours after its injection, with a return to normal in 12 hours and a subsequent rise above normal for the remainder of the experiment (fig. 1, curve E). Male and female animals, 38 days of age, were employed.

Epiphyses. (*a*) *Progesterone.* In the epiphyses of the femurs of the same rats, the 5.0 mg. dose of progesterone produced no regular or marked change in the phosphatase content (fig. 1, curve F).

(*b*) *Testosterone.* The same quantity of testosterone, however, caused a marked increase of the phosphatase which was still evident 48 hours after the injection (fig. 1, curve G).

(*c*) *Estradiol.* The phosphatase content of the epiphyses following the administra-

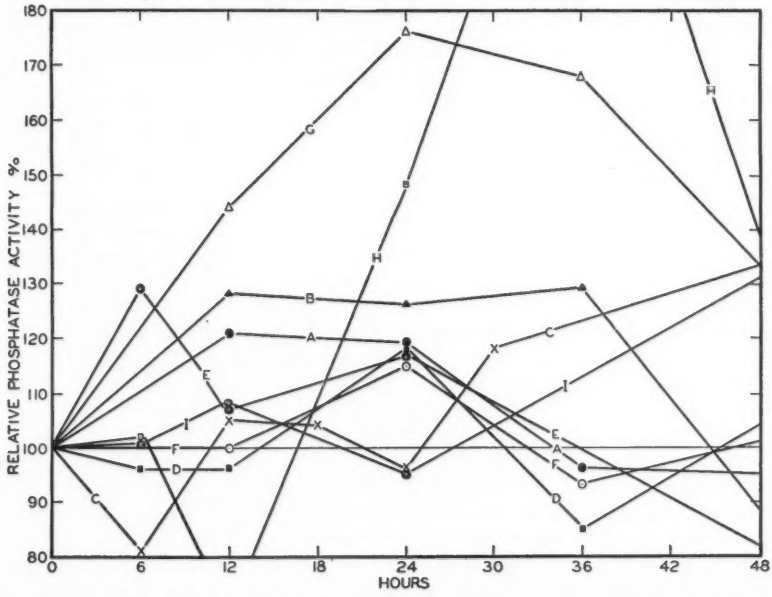


Fig. 1. Effects of the sex hormones, thyroxin and thymus extract on the phosphatase content of the femurs of rats. *Diaphyses:* A, 5.0 mg. progesterone; B, 5.0 mg. testosterone; C, 1.5 mg. estradiol; D, 1.0 mg. thyroxin-sodium; E, 2.0 cc. thymus extract. *Epiphyses:* F, 5.0 mg. progesterone; G, 5.0 mg. testosterone; H, 1.0 mg. thyroxin-sodium; I, 2.0 cc. thymus extract.

tion of the estradiol was quite variable, the results ranging from one-half to three times the normal values. On the average, however, a slight increase above the normal was apparent (not charted).

(*d*) *Thyroxin.* The injection of the thyroxin-sodium produced a marked increase of the epiphyseal phosphatase, with a return of the values towards the normal in 48 hours (fig. 1, curve H).

(*e*) *Thymus extract.* The thymus extract brought about an irregular variation of the phosphatase about the normal, reaching a moderately high value 48 hours after its injection (fig. 1, curve I).

DISCUSSION

Although considerable confusion exists with regard to the role of the hormones in osseous metabolism, published data indicate that the sex hormones influence, directly or indirectly, the structure and composition of bone.

While the present investigations did not include histologic studies, attempts have been made to correlate the biochemical findings with the cytologic observations of others. Silberberg and Silberberg (1) found that the administration of progesterone to guinea pigs for periods of 1 to 8 weeks promoted the proliferation and hypertrophy of the epiphyseal and articular cartilage cells and inhibited the retrogressive changes which are characteristic of the aging process. These findings were almost the opposite of the acceleration of aging which they observed in the skeletal tissues following the injection of estrogen (2). According to Roche, Filippi and Leandri (3), the role of phosphatase in osseous metabolism is more closely associated with the proliferation of the organic matrix of bone than with the actual process of mineralization.

According to circumstances, including the dosages employed, the androgenic substances apparently can stimulate (4, 5, 6, 7) or depress (8, 9) the somatic growth of animals. Augmentation of the process of ossification has been observed as a result of the long-continued administration of the androgens (6).

Clinically, Browne (10) found that the injection of testosterone propionate to a sexually immature man caused an increase of stature of one-half inch in a few months. Rapfogel (11) obtained an increase of stature in excess of the expected normal in a $15\frac{1}{2}$-year-old eunuch following treatment with testosterone propionate. Likewise, Webster and Hoskins (12) reported that the administration of testosterone propionate to 8 hypogonadal adolescent boys was accompanied by an increase in average growth rate. Following the cessation of treatment, the average rate of growth again fell almost to that of the preliminary control period.

These therapeutic effects may be related to the marked increase of the phosphatase content of the bones, especially obvious in the epiphyses, which we found after the administration of testosterone. It has been shown by Roche, Filippi and Leandri (3) that an increase in the phosphatase is associated with an increased rate of growth of the bones.

In general, the results which we obtained following the injection of estradiol may be correlated with certain experimental data. For example, the increased phosphatase content of the femurs may be related to the increased size and number of the centers of ossification in immature female rats as shown by Talbot (13). Pfeiffer and Gardner (14) observed hypercalcification of the marrow-containing bones of pigeons receiving estrogen. The irregular character of the results which we obtained may correspond with the variations in response observed by Gardner (15) in the pelves of mice following the administration of estrogenic substances. Apparently, under such conditions, both the resorption and calcification of bone may go on side by side. Day and Follis (16) found the bone phosphatase to increase following the treatment of 30-day-old rats with estradiol benzoate. The effect became less marked with advancing age and the prolongation of the period of treatment.

During the healing of fractures, the bone phosphatase has been found to be increased (17). Pollock (18) noted that the spaying of rats inhibited the healing of induced fractures and that the administration of theelin facilitated their repair.

Our results with thyroxin are in agreement with those of Scoz and Marangoni (19) who found that it increased the phosphatase activity of the bones of rats. It is conceivable that some correlation may exist between these observations and the histological studies of Silberberg and Silberberg (20), who found that the initial effect of thyroxin consisted of proliferative and hypertrophic changes in the epiphyseal and articular cartilages of mice, followed by retrogressive processes.

SUMMARY

Progesterone, testosterone and estradiol tend to increase the phosphatase content of the femurs of rats.

The effect is especially pronounced in the epiphyses following the administration of testosterone. This observation may provide an explanation for the increased rate of growth of human beings such having been reported to follow the administration of testosterone propionate.

Thymus extract causes a moderate increase of the bone phosphatase.

The observations of others with regard to the ability of thyroxin to increase the phosphatase content of bone have been confirmed.

No conclusions can be drawn with regard to the mechanism of these changes.

REFERENCES

1. Silberberg, M., and R. Silberberg: *Arch. Path.* 31: 85. 1941.
2. Silberberg, M., and R. Silberberg: *Arch. Path.* 28: 340. 1939.
3. Roche, J., A. Filippi and A. Leandri: *Bull. Soc. Chim. Biol.* 19: 1314. 1937.
4. McEuen, C. S., H. Selye and J. B. Collip: *Proc. Soc. Exper. Biol. & Med.* 36: 390. 1937.
5. Bottomley, A. C., and S. J. Folley: *J. Physiol.* 94: 96. 1938.
6. Sainton, P., H. Simonnet and J. Petrescu: *Compt. Rend. Soc. de Biol.* 128: 698. 1938.
7. Rubinstein, H. S., and M. L. Solomon: *Endocrinology* 28: 229. 1941.
8. Kochakian, C. K.: *Endocrinology* 26: 54. 1940.
9. Rubinstein, H. S., A. A. Kurland and M. Goodwin: *Endocrinology* 25: 724. 1939.
10. Browne, J. S. L.: *Personal communication.*
11. Rappogel, I.: *Endocrinology* 27: 179. 1940.
12. Webster, B., and W. Hoskins: *Proc. Soc. Exper. Biol. & Med.* 45: 72. 1940.
13. Talbot, N. B.: *Endocrinology* 25: 325. 1939.
14. Pfeiffer, C. A., and W. U. Gardner: *Endocrinology* 23: 485. 1938.
15. Gardner, W. U.: *Am. J. Anat.* 59: 459. 1936.
16. Day, H. G., and R. H. Follis, Jr.: *Endocrinology* 28: 83. 1941.
17. Roche, J., and A. Filippi: *Bull. Soc. Chim. Biol.* 20: 1147. 1938.
18. Pollock, C. A.: *Proc. Staff Meet. Mayo Clin.* 15: 209. 1940.
19. Scoz, G., and P. L. Marangoni: *Bull. Soc. Ital. Biol. Sper.* 9: 969. 1934.
20. Silberberg, M., and R. Silberberg: *Growth* 4: 305. 1940.

RELEASE OF GLUTATHIONE INHIBITION OF MELANIN FORMATION BY ESTRONE

FRANK H. J. FIGGE[1] AND EDGAR ALLEN

From the Departments of Anatomy, Yale University School of Medicine, and the University of Maryland Medical School

NEW HAVEN, CONNECTICUT AND BALTIMORE, MARYLAND

THE FACT that pigmentary changes occur in man coincident with functional alterations in sex organs was observed long ago. The pigmentary change in the nipple and areola during pregnancy affords a well-known example. Likewise, injection, transplantation, castration and tissue culture experiments indicate that sex hormones may influence secondary sex or even general pigmentation (1–9). This subject was recently reviewed by Domm, Danforth, and Moore (10) where additional references may be found. In many of the cases recorded, it is melanin that is partially or entirely responsible for the pigmentary changes. It is hoped that the observations recorded in this report may eventually lead to more definite knowledge of the mechanism by which these endocrine substances influence the process of melanin formation.

It had been demonstrated that reduced glutathione inhibits melanin formation in buffered tyrosine-tyrosinase mixtures (11, 12). When the glutathione is completely oxidized, melanin formation takes place at a rate comparable to that in the control (12). The oxidation of the glutathione by atmospheric oxygen in such a mixture is accelerated by the excess copper present in the enzyme preparation (13–18). The inhibition of melanin formation by glutathione is thus reversible and temporal (12). The length of the inhibitory period depends upon the amount of reduced glutathione added (11, 12). Cysteine functions in a similar manner (11).

An attempt was made to find a substance or group of substances that would release the glutathione inhibition. Several substances such as oxidized methylene blue and toluyene blue were found to do this. Estrone was included in one of the series of experiments and was found to release the glutathione inhibition. The result with estrone was so unexpected and striking that this was investigated further and forms the basis of the present paper.

METHODS AND MATERIALS

Tyrosine was dissolved by adding 2 cc. of 0.2 M sodium hydroxide and then sufficient water to give a 1.5×10^{-3} M solution. The addition of one-half volume of buffer, enzyme and other components, changed this to 1×10^{-3} M (3.6 mg. per 20 cc.). The concentrations of the other stock solutions of substances were calculated so that the concentrations in the reaction mixtures could be expressed in terms of final molar concentration.

The buffer solution used was Clark's sodium hydroxide phosphate buffer, pH 6.8. The final adjustment was made with the aid of a glass electrode pH meter. Each individual reaction mixture was also checked and adjusted to make certain that the

[1] Rockefeller Fellow, 1940–41.

pH was uniform. This was necessary because estrone,[2] which was added in varying amounts in relatively high concentrations had to be dissolved in a hydroxide-alcohol solution. This was first dissolved in 2 cc. of 0.2 M sodium hydroxide plus 2 cc. of ethyl alcohol. Water was then added to give the desired molarity. The glutathione was dissolved in water immediately before use. The enzyme preparation was fresh fast filtered potato juice diluted with 4 volumes of buffer solution. One cc. of this was added to each reaction mixture. Controls using boiled enzyme were used in the early experiments.

The substances were added to the reaction bottles (70 cc. wide-mouth) in the following order: tyrosine, estrone, buffer, glutathione and finally enzyme. The final volume in each reaction bottle was 20 cc. The light absorption was determined at once by means of a photo-electric colorimeter, using a monochromatic filter with a transmission band at 0.570μ. To follow the rate of melanin formation similar readings were made at 1- and 2-hour intervals. The change in light absorption in any given period was taken as an indication of the amount of melanin formation in that period.

Four control reaction mixtures were always included; two preceding and two following each experimental series. These contained only tyrosine, buffer and enzyme.

To test the effect of estrone on this reaction, 5 concentrations were used: 40×10^{-5} M; 20×10^{-5} M; 10×10^{-5} M; 6×10^{-5} M; and 2×10^{-5} M (2.0; 1.0; 0.5; 0.3; 0.1 mg. per 20 cc. respectively). At the higher concentrations a fine colloidal precipitate appears when the buffer is added. This does not seem to destroy the influence of the estrone. Glutathione was added to another series in the following concentrations: 100×10^{-5} M; 60×10^{-5} M; 40×10^{-5} M; 20×10^{-5} M; and 10×10^{-5} M (6.0; 3.6; 2.4; 1.2; 0.6; mg. per 20 cc. respectively).

In a third series, both glutathione and estrone were added to each bottle. The concentrations listed above were likewise used in this series. However, the first reaction bottle of the series contained the high concentration of estrone plus the low concentration of glutathione. In succeeding bottles, the concentration of glutathione increased while the estrone concentration decreased. The molecular concentration ratios of estrone as compared to glutathione were as follows: 4:1; 1:1; 1:4; 1:10; 1:50.

RESULTS

The results of one of the experiments were tabulated as shown in table 1. The highest concentrations of estrone (2.0 and 1.0 mg. per 20 cc.) caused marked inhibition of melanin formation while the lowest concentration used in this experiment (0.1 mg. per 20 cc.) did not alter the rate of melanin formation by tyrosinase. Lower concentrations (0.05–0.005 mg. per 20 cc.) in other experiments had an accelerating effect on the rate of melanin formation by tyrosinase. The melanin which formed in the presence of estrone differed from ordinary synthetic melanin in that it was not jet black, but was reddish brown in color. This color may be due to a reduced melanin and a red colored oxidation product of estrone.

The fact that reduced glutathione inhibited the rate of melanin formation by tyrosinase was mentioned in previous papers (11, 12). The concentrations in this experiment were higher than those used in previous experiments. The three highest concentrations inhibited melanin formation completely for 7.5 hours. Some melanin formed even in these mixtures for at the end of 22 hours, the light absorption values were recorded as 2.8; 29.3; and 46.6%. In the lower concentrations, the inhibitory effect terminated at the end of 1 and 5 hours as indicated. When these values were

[2] Estrone was supplied by The Schering Corporation, Bloomfield, N. J., through the courtesy of Dr. Erwin Schwenk.

plotted against time, it was apparent that the inhibition was only a temporal one. When the glutathione was completely oxidized and the reaction started, the slope of the curve was essentially the same as for the control. The glutathione thus merely caused a delayed reaction. The extent of the delay was determined by the amount of glutathione added.

In contrast, the type of inhibition caused by high concentrations of estrone was evident and uniform from the beginning to the end of the reaction. When these values for melanin formation in the presence of estrone were plotted, the curves all radiated from the point of origin, and the slopes which indicate the rate of melanin formation

TABLE 1. THE INFLUENCE OF ESTRONE AND GLUTATHIONE ON THE RATE OF MELANIN FORMATION IN TYROSINE-TYROSINASE MIXTURES

Reaction mixture No.	Substances added, mg. per 20 cc.		Melanin concentrations as indicated by percentage light absorption at end of:			
	Estrone	Glutathione	1 hr.	4 hr.	6 hr.	7½ hr.
1			3.3	22.5	34.9	39.6
2			3.8	26.2	39.7	45.0
3	2.0		0.0	4.0	8.4	11.0
4	1.0		2.0	11.0	20.0	23.0
5	0.5		1.8	17.0	27.2	31.0
6	0.3		1.2	14.0	28.8	34.4
7	0.1		2.3	26.5	40.5	42.5
8		6.0	0.0	0.0	0.0	0.0
9		3.6	0.0	0.0	0.0	0.0
10		2.4	0.0	0.0	0.0	0.0
11		1.2	0.0	0.0	2.1	10.4
12		0.6	0.1	9.0	24.0	31.7
13	2.0	0.6	0.2	6.2	10.3	13.2
14	1.0	1.2	0.0	9.0	16.8	20.2
15	0.5	2.4	0.0	0.5	0.8	1.0
16	0.3	3.6	0.0	0.0	0.5	1.6
17	0.1	6.0	0.0	0.0	0.0	0.0
18			2.5	25.1	37.5	43.5
19			1.5	23.2	36.0	43.0
Average of controls			2.8	24.3	37.0	42.8

Substrate.....tyrosine 3.6 mg. per 20 cc.
Buffer.....phosphate-sodium hydroxide
pH.....6.8
Enzyme.....potato tyrosinase (0.2 cc. plus 4 volumes buffer)
Temperatures.....25°–26° C.

varied with the concentration. When the rate of melanin formation in reaction mixture No. 13 (see table 1) was compared with its corresponding controls, No. 3 and No. 12, it was seen that the rate was intermediate, higher than that in No. 3 and lower than in No. 12 as would be expected from the fact that the molecular ratio was 4 estrone to 1 of glutathione; inhibition was of the type characteristic of estrone.

In only one of the reaction bottles (No. 14) containing both estrone and glutathione was the release of the glutathione inhibition very marked. This was in the mixture containing the two substances in equimolecular concentration (20×10^{-5} M or 1.2 mg. glutathione 1.0 mg. estrone per 20 cc.). It was found that the rate of melanin formation in this mixture paralleled the rate in the corresponding estrone control. When this was observed in the first experiment, it was thought that the glutathione

had been inadvertently omitted from this one reaction mixture. The experiment was repeated 4 times in precisely the same way and special precautions were taken to check the addition of glutathione, as it was added to the reaction mixture. In each case, the single reaction mixture in the series, containing both estrone and glutathione in equimolecular concentration, stood out above the others in the rate of melanin formation.

This effect was clearly shown by a graph (fig. 1) in which the rate of melanin production in mixture No. *14* was compared with that in No. *4* and No. *11*. The rate was significantly higher than in mixture No. *11* and very slightly lower than in No. *4*.

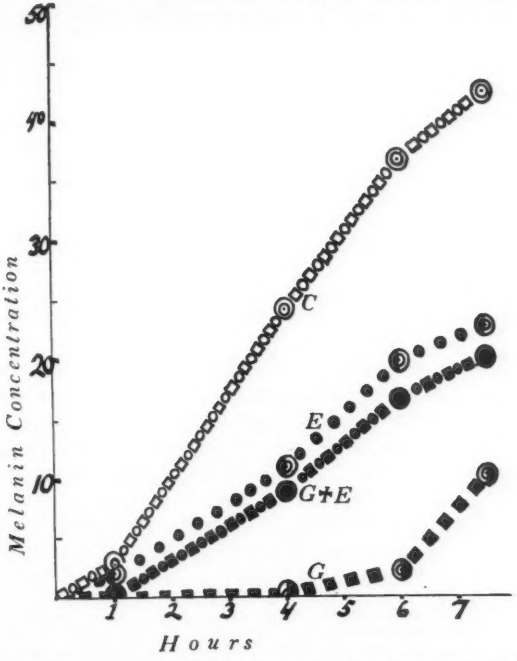


Fig. 1. THE RATE OF MELANIN FORMATION IN TYROSINE-TYROSINASE MIXTURES. pH 6.8, temperature 26°. *Curve C*—control 10^{-3} M (3.6 mg.) tyrosine plus 0.2 cc. potato juice enzyme. (Reaction mixture average of No. *1*, *2*, *18*, *19*.) *Curve G*—influence of 20×10^{-5} M (1.2 mg. per 20 cc.) glutathione. (Reaction mixture No. *11*.) *Curve E*—influence of 20×10^{-5} M (1 mg. per 20 cc.) estrone. (Reaction mixture No. *4*.) *Curve G+E*—influence of 20×10^{-5} M glutathione plus 20×10^{-5} M estrone. (Reaction mixture No. *14*.)

The slopes of the curves represent the rate of melanin formation in reaction mixtures Nos. *3*, *11* and *14*. Melanin concentration or percentage light absorption was plotted along ordinates and the time along abscissae. The slope in curve "C" represents the average rates in 4 control reaction mixtures containing only buffered tyrosine and tyrosinase (Nos. *1*, *2*, *18*, *19*). Curve "E" represents the rate in a similar mixture with the addition 1.0 mg. estrone (No. *4*). Curve "G" shows the inhibition (complete for approximately 5 hours) caused by 1.2 mg. glutathione (No. *11*). Curve "G+E" shows that the inhibitions produced by these two substances are not additive, but on the contrary, that estrone when added in equimolecular concentration released the glutathione inhibition in one hour. After this time the slope of the curve was essentially the same as that in curve "E."

DISCUSSION

That estrone can indirectly influence an enzyme reaction that is regulated by the oxidation-reduction potential of substances added to the reaction mixture (11) may be significant. The reactions that occur when estrone is added to a tyrosine-tyrosinase glutathione mixture are not known. It was suggested[3] that the enzyme oxidizes ring A of estrone to a quinone and that this (in the presence of copper catalyst) rapidly oxidizes the glutathione and thus releases the inhibition. The first part of this reaction has been postulated to explain the inactivation of estrogens by tyrosinase (18). If such is the case, the quinone so formed would probably be a reversibly oxidizable substance and its influence would doubtless depend on its oxidation-reduction potential and rate of reactivity with glutathione.

The fact that the pigment which forms in tyrosine-tyrosinase mixtures in the presence of estrone is not jet black, but red to brown in color is of interest in view of Hamilton's observations (9). He found that the addition of estrone and other sex hormones to the culture media in which he explanted pieces of skin from 6-day old Rhode Island Red chicks caused the development of large numbers of red melanophores. The control explants favored the development of black melanophores and red ones differentiated rarely.

SUMMARY

Estrone in concentration higher than 0.3 mg. per 20 cc. partially inhibited the formation of melanin in a tyrosine-tyrosinase mixture. The degree of inhibition was dependent on the concentration.

The pigment or pigments which formed in such mixtures appeared reddish brown in contrast to the jet black pigment formed in mixtures without estrone. The color may be due to a mixture of reduced melanin and a red oxidation product of estrone.

Glutathione (1.2 mg. per 20 cc.) completely inhibited the formation of melanin by tyrosinase for a period of 5 hours. When estrone and glutathione were added in equimolecular quantities (1 mg. estrone and 1.2 mg. glutathione) to a tyrosine-tyrosinase mixture, the estrone released the enzyme from the glutathione inhibition in one hour.

REFERENCES

1. Juhn, M., and R. G. Gustavson: *Anat. Rec.* 44: 204. 1929.
2. Keck, W. N.: *J. Exper. Zool.* 67: 315. 1934.
3. Danforth, C. H.: *Proc. Soc. Exper. Biol. & Med.* 33: 291. 1935.
4. Danforth, C. H.: *Proc. Soc. Exper. Biol. & Med.* 36: 322. 1937.
5. Danforth, C. H.: *Anat. Rec.* 68: 461. 1937.
6. Witschi, E.: *Proc. Soc. Exper. Biol. & Med.* 35: 484. 1936.
7. Hamilton, J. B.: *Proc. Soc. Exper. Biol. & Med.* 40: 502. 1939.
8. Hamilton, J. B., and G. Hubert: *Science* 88: 481. 1938.
9. Hamilton, H. L.: *Proc. Soc. Exper. Biol. & Med.* 45: 571. 1940.
10. Allen, E., C. H. Danforth and E. A. Doisy: Sex and Internal Secretions. Williams & Wilkins, Baltimore. 1939.
11. Figge, F. H. J.: *J. Cell. & Comp. Physiol.* 15: 233. 1940.
12. Figge, F. H. J.: *Proc. Soc. Exper. Biol. & Med.* 46: 269. 1941.
13. Kubowitz, F.: *Biochem. Ztschr.* 296: 443. 1938.
14. Elliot, K. A. C.: *Biochem. J.* 24: 310. 1930.
15. Elvehjem, C. A.: *Biochem. J.* 24: 415. 1930.
16. Rosenthal, S. M., and C. Voegtlin: *Pub. Health Rep.* 48: 347. 1933.
17. Kharasch, W. S.: *J. Biol. Chem.* 113: 537. 1936.
18. Westerfeld, W. W.: *Biochem. J.* 34: 51. 1940.

[3] We are indebted to Dr. Ralph J. Dorfman for this and other suggestions.

THE REPRODUCTIVE ORGANS IN MALNUTRITION

EFFECTS OF CHORIONIC GONADOTROPIN UPON ATROPHIC GENITALIA OF UNDERFED MALE RATS

MICHAEL G. MULINOS AND LEO POMERANTZ

From the Department of Pharmacology, College of Physicians and Surgeons, Columbia University

NEW YORK CITY

PROLONGED UNDERFEEDING, whether induced intentionally, or incidentally as the result of some extraneous experiment which causes anorexia, leads to marked depression of the endocrine glands (1). It has been suggested that the term pseudo-hypophysectomy best describes the regressive changes in the endocrine organs occurring during inanition, because they resemble the changes which follow hypophysectomy (1–4). Some evidence in support of the validity of this term comes from the work of Mason and Wolfe (5) who reported that the hypophyses of underfed male rats had low gonadotropic activity. Werner (6) corroborated these findings by demonstrating a decrease in the gonadotropic hormone content of the hypophysis of the underfed female rat. More recently the pseudo-hypophysectomy syndrome was studied in the chick (7, 8) in the guinea pig (9) and in man (10).

Jackson (11) reviewed the literature on the effects of inanition on the testes and accessory genitalia. The major portion of the anatomical data was reported by Siperstein (12). Mason (13) discussed the endocrine etiology of the atrophy of the testes during inanition. However, few data are available on the effects of hormones upon the male genital tract during inanition. Moore and Samuels (14) using underfed male rats were able to induce hypertrophy of the accessory genitalia by the injection of testis hormone, and in several rats, by means of a pregnancy urine extract.

The purpose of this report is to present further studies on the effects of underfeeding upon the male genital organs and the effects of the gonadotropic substance from pregnancy urine upon the testes and upon the accessory genitalia of underfed male rats.

EXPERIMENTAL

The experiments were performed on 2-month-old male albino rats of the Sherman strain weighing approximately 200 gm. Twenty-two rats were used in the first series of experiments (table 1). Eight were chronically underfed by placing each on a diet containing one-half of the usual amount of stock rations ingested during control observations. Under this regime, the rats lost approximately one-third of their body weight in 30 days, at which level they remained. The other 14 rats were allowed to feed freely for almost 2 months. During this time, their weights rose from 197 to 275 gm. Seven rats of this group were then starved completely for 7 days, at the end of which time all of the 22 rats were autopsied.

In the second series of experiments outlined in table 2, 35 rats were used. Ten of

Received for publication June 23, 1941.

these were autopsied in order to obtain pre-inanition organ weights. Twenty-five were underfed for from 43 to 65 days. In order to determine the condition of the genitalia prior to the injection of chorionic gonadotropin (CG),[1] the left testis and epididymis were removed surgically from 23 of the rats and 2 were autopsied as controls. Three of the unilaterally castrated rats were allowed food ad libitum and were kept as uninjected controls for the underfed group as a test for the recuperative power of the remaining testis and accessory genital organs. The remaining 20 rats continued to be underfed; 5 received daily (except Sunday) subcutaneous injections of 25 R.U. of the CG; 5 received 15 R.U.; 5 received 10 R.U.; and 5 were not injected.

TABLE 1. EFFECTS OF CHRONIC UNDERFEEDING AND OF COMPLETE STARVATION UPON THE GENITALIA OF UNDERFED MALE RATS

State of nutrition	No. of rats	Days observed	Body weight			Testes	Seminal vesicles	Prostate	Epididymis	Rest of genitalia	Hypophysis
			Initial	Before starvation	Final						
			gm.	gm.	gm.	mg.	mg.	mg.	mg.	mg.	mg.
Chronic Underfeeding											
a)	5	61	196	—	145	2,030	180	300	540	820	5.4
b)	3	65	193	—	133	730	70	80	230	600	4.8
Complete starvation for 7 days	7	62 (7 starved)	198	277	183	2,570	580	530	690	1,340	7.4
Normally fed	7	62	196	—	263	2,850	910	650	910	1,620	8.2

Every day during the period of observation, the scrotum of each rat was palpated lightly and records kept by means of symbols. A + + + testis was considered normal and upon palpation felt large and turgid and was placed well down in the scrotum. A + + testis was one that was either smaller or less turgid than normal. A testis was considered + if it were notably atrophic. If any one of these testes was retracted, it was moved back into the scrotum by gentle pressure. The testis was then palpated and recorded as usual for size but with a zero exponent to denote the retracted position. If a retracted testis could not be easily moved into the scrotum, the fact was recorded as a 0 testis. The testes and epididymi which were removed at the biopsy operations were weighed immediately and fixed in Zenker's fluid. The rats, one from each group, were autopsied after 20, 23, 27, 30 and 44 days of treatment with the CG. Routine histological preparations were stained with hematoxylin and eosin.

The area of the testicular tubules was determined from a stained section, the image of which was projected onto a glass plate. Twenty representative circular tubules were traced upon a sheet of paper held against the glass plate and the areas measured by means of a planimeter. The difference in tubule area of the testes at biopsy and at autopsy gave a measure of the effectiveness of the hormonal injections (table 2, fig. 1).

RESULTS

Effects of inanition on the testes. Complete starvation for 7 days lowered the weight of the testes to slightly below the controls (table 1), and this fall was probably due to a decrease in the fluid content of the tubules, since, histologically, tubular changes were slight or absent.

The atrophy of the testes from chronic inanition was variable in degree. The testes

[1] The chorionic gonadotropin (Follutein) was supplied by E. R. Squibb and Sons, New York City.

TABLE 2. EFFECTS OF CHORIONIC GONADOTROPIN UPON THE GENITALIA OF UNDERFED MALE RATS[1]

Group	No. of rats	Body weight			Days underfed before operation	Procedure after operation	Days of further underfeeding	Total CG per group	Testis weight		Testis tubule area		Epididymis weight		Seminal vesicles	Prostate	Rest of genitalia
		Initial	Before treatment	After treatment					Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment			
		gm.	gm.	gm.				R.U.	mg.	mg.	Sq. mm. × 10^{-2}		mg.	mg.	mg.	mg.	mg.
Control	10	199	—	199	0	—	—	—		1,070[2]	—	5.02		253[2]	363	346	970
69 A	2	198	—	130	51[3]	—	—	—		400[2]	—	—		112[2]	100	50	570
65	5	201	133	137	58	Continued underfed	35	—	590	450	2.89	2.36	146	109	65	51	480
66	5	204	136	133	64	25 R.U. per day	29	3,025	240	840	1.95	3.73	91	204	914	540	1,150
67	5	206	136	136	64	Continued underfed 15 R.U. per day	29	1,815	300	790	2.08	3.55	102	224	1,252	540	1,240
68	5	202	137	135	65	Continued underfed 10 R.U. per day	29	1,210	360	930	2.19	3.58	113	246	1,380	720	1,380
69	3	210	133	239	65	Continued underfed Refed 28 days	—	—	490	980	2.62	4.62	138	195	290	310	900

[1] Figures are averages of results obtained after varying periods of treatment.
[2] One-half of the weight of both testes or epididymi.
[3] No operation.

of some rats became severely atrophic whereas others underfed to the same extent, had testes approximately normal in weight. However, it was usual that the rats which had lost the most weight also showed the greatest testicular atrophy. These animals also had the most atrophic pituitary glands.

In other experiments, some rats have been underfed for several months, at the end of which time the testes were normal in weight, and on section appeared normal except for atrophy of the interstitial tissue. Other underfed rats presented testes which fluctuated slightly in size, decreasing to about a ++, then returning to normal.

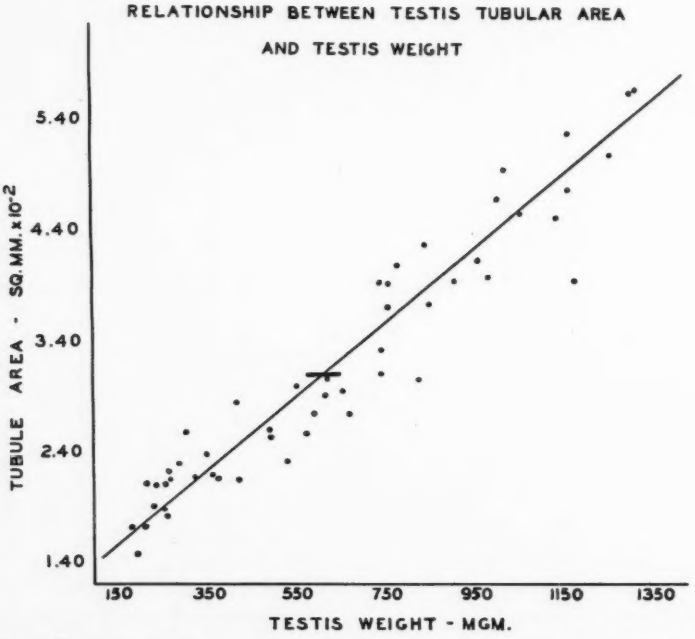


Fig. 1. The relationship between tubule area and testis weight of rats, regardless of the state of nutrition or treatment. The chart reveals that a large testis is composed of large tubules and not of many small ones. The dots below the bisecting bar are from the left testes mainly of underfed untreated rats, those above the bar are from the right testes of the underfed rats but after treatment with chorionic gonadotropin or after refeeding. Several normally fed untreated rats are also represented above the bar.

However, when a testis had decreased in weight as much as those reported in table 2, it remained in that depressed state as long as the undernutrition persisted. Consequently, one cannot evaluate the effectiveness of gonadotropic hormone preparations upon the germinal epithelium of the underfed rat unless, prior to treatment, spermatogenesis is absent, the tubules are markedly atrophic and there is no evidence of a fluctuating testis size. Despite the presence of evidently normal tubules and of spermatogenesis in the testes of some underfed rats, there was always atrophy of the interstitial cells and accessory genital organs. It is possible that the pituitary hormones responsible for tubular growth and for interstitial cell activity are not affected equally by the inanition.

In a second series of experiments on chronic inanition, the fluctuation in the size

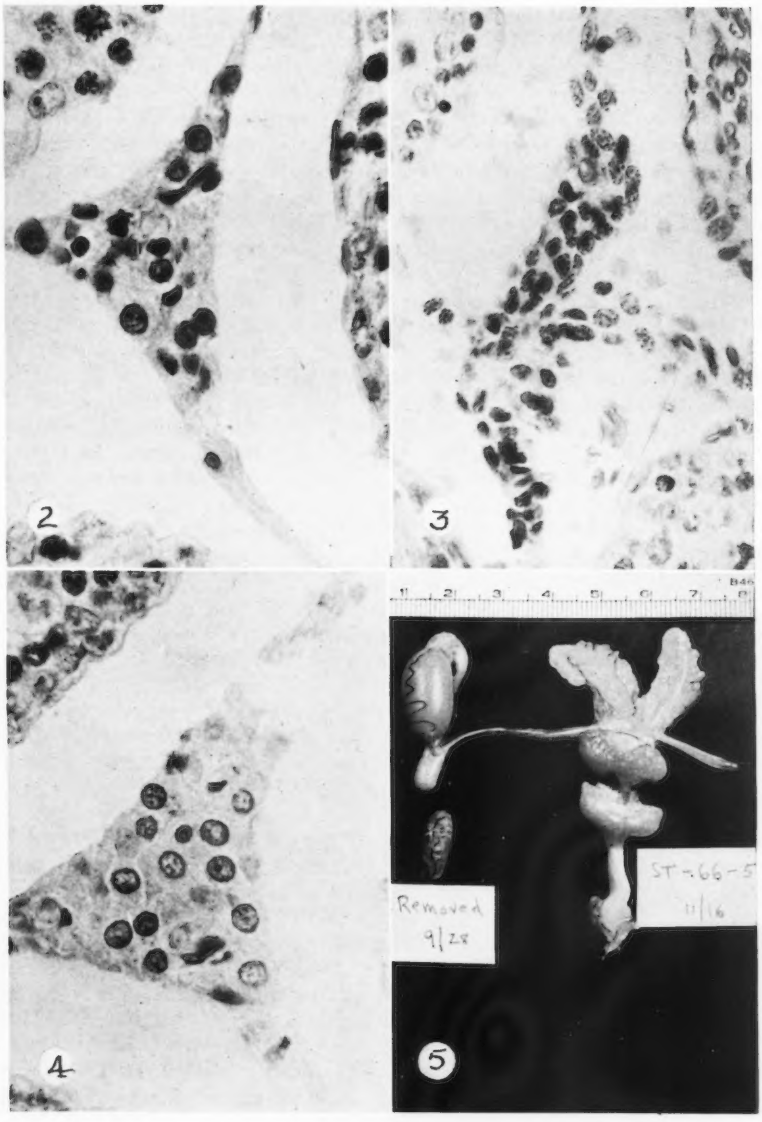


Fig. 2. INTERSTITIAL TISSUE OF TESTIS OF A NORMALLY FED CONTROL RAT. Note moderate amount of cytoplasm and size of nuclei. Fig. 3. A LARGE ISLAND OF INTERSTITIAL TISSUE FROM THE LEFT TESTIS SURGICALLY REMOVED FROM A CHRONICALLY UNDERFED RAT, SHOWING THE WHEEL-CELL FORMATION OF THE NUCLEAR CHROMATIN. Fig. 4. INTERSTITIAL TISSUE FROM RIGHT TESTIS OF THE SAME RAT AS IN FIGURE 3, AFTER 44 DAYS OF TREATMENT WITH CHORIONIC GONADOTROPIN (a total of 875 R.U.). Note the large nuclei with absence of wheel-cell formation, and the increase in the amount of cytoplasm. (photomicrographs ×630) Fig. 5. At lower left is shown the left testis surgically removed (weight, 280 mg.) after 64 days of chronic underfeeding. The rest of the figure shows the genitalia at autopsy after 44 days of treatment with chorionic gonadotropin (a total of 875 R.U.) and continued underfeeding (right testis weight, 1,140 mg.).

of the testes became less marked as the period of inanition progressed. Of the 25 rats underfed for 36 days, 8 had +++ or apparently normal testes; 9 had ++; and 8 had + testes. None was abdominal. Surgical removal of the left testis was performed on the 58th to the 65th day of inanition, at which time none of the rats had normal testes, 5 were ++; and 18 were +. Three to 6 weeks after the biopsy, 3 of the untreated rats had ++ testes, one had + testis, and one had a 0 testis, as compared to the testes of the 15 rats treated with CG of which 5 were +++, 9 were ++, and one was +. The testes of the rats at biopsy varied in weight from 0.25 to 0.59 gm., and in tubule area from 0.0142 to 0.0390 sq. mm. The testis size estimations made by palpation were well correlated with the actual weight of the testes made after biopsy or later at autopsy.

The microscopic appearance of the atrophic testes was similar to that reported by Siperstein (12) and Jackson (11). The spermatozoa disappeared first, followed by desquamation of the spermatids and spermatocytes. Mason (13) pointed out that some of the desquamated spermatids may be carried to the epididymis where they undergo further degeneration. This was true also in our experiments, where it was noted that spermatids appeared in the ductus epididymis, indicating that the obscure mechanism which is responsible for the conveyance of tubular elements into the epididymis does not disappear during inanition. Later, when the testes became maximally atrophied and microscopic section revealed but a few desquamated cells within the tubules, the epididymis was shrunken, markedly atrophic, and free of formed elements.

Effects of chorionic gonadotropin on the testes during chronic inanition. Within 2 weeks after the CG injections were begun, palpation revealed enlargement of the previously atrophic testes. After 3 weeks of treatment, histological section revealed definite growth and development of the seminiferous epithelium up to the spermatid stage. After 30 days of treatment, autopsy revealed no spermatozoa in the testes, but numerous spermatids were found in the epididymi. Regardless of the dose, only after 44 days of treatment with CG (10 to 25 R.U. daily) did the testes again contain spermatozoa. On the other hand, only 20 days of refeeding were necessary for the appearance of spermatozoa in the epididymi of previously undernourished rats.

Despite the fact that there occurred comparable growth in size and weight of the testes, of both the refed and the CG injected rats, there was a much greater lag in the reappearance of spermatozoa in the testes of the CG injected animals. The delay in spermatogenesis in response to CG during continued inanition may have been due to the dietary deficiency of underfeeding or to an inadequate hormone production referable to the 'pseudo-hypophysectomy' induced by the inanition. Smelser (15) found that removal of one testis in the normally fed rat resulted in compensatory hyperactivity of the germinal epithelium of the remaining testis. During chronic inanition our rats showed no hypertrophy of the remaining testis, although it was presumably acted upon by all available gonadotropic hormone. Actually, the remaining testis continued to atrophy and lost an additional 22% in weight and 16% in tubule area between the time of operation and autopsy (20 to 44 days). These figures are within the range of testicular atrophy from inanition in rats in which both testes were present throughout the experimental period.

Table 2 summarizes the averaged effects of CG and refeeding upon the testis remaining after the biopsy removal of the other.

The detailed data show the following.

(*a*) That after 40 daily injections of CG the testes enlarged to a common figure (1.07 to 1.18 gm.) which was the same as that obtained after refeeding.

(*b*) That there is a dose-time relationship in the response of the testis to CG or to refeeding. Twenty-five R.U. of CG given for 20 days do not result in the maximal

growth which is obtained from 10 R.U. given for 43 days. A total dose of 425 R.U. over 20 days resulted in an average testis weight of 0.74 gm., while a total of 350 R.U. over 43 days resulted in a testis of normal weight (1.18 gm.).

(c) That as a result of injections of CG or from refeeding, the rate and degree of testicular growth are dependent upon the severity of the initial atrophy due to the underfeeding. A rat refed for 23 days showed a gain in testis weight of from 0.75 gm. at biopsy, to 1.24 gm. at autopsy (+65%), while another, which was refed for 43 days, showed a change of from 0.36 to 1.07 gm. (+193%). Percentile changes in the weight of testes which do not weigh the same at biopsy are of dubious significance because the weight-growth curve of the testis is asymptotic.

(d) That although daily doses of 25 R.U. of CG were no more effective in eliciting spermatogenesis than were doeses of 10 R.U., they did cause a greater percentile increase in testis weight and cross-section tubule area (table 2).

Effects of inanition on the accessory genitalia. In complete starvation for 7 days, the weight loss of the accessory genitalia (table 1) is in large part dependent upon the decreased secretions within the lumina of these organs. Thus, the seminal vesicles, which normally contain the most secretion, lose more weight than any of the other accessory organs.

During chronic inanition, the accessory sex organs atrophied in a manner approximately proportional to the degree and duration of the inanition (table 1), but the percentile loss in weight was greater than the loss in body weight (1). The shrinkage of the accessories usually occurred before the testes were palpably reduced in size and resembled the atrophy which occurs in castrated rats (14). The seminal vesicles and the prostate gland were most severely affected. In extreme cases the seminal vesicles were decreased in weight from the average normal of 363 mg. to 45 mg., and the prostate decreased from 345 mg. to 20 mg. After 58 to 65 days of underfeeding, the weight of the left epididymis, removed at operation, averaged 118 mg. (70 to 220) as compared to an average of 254 mg. (145 to 370) for the normal rat. The weight of the normal epididymis is maintained by the numerous germ elements flowing into it from the testis as well as by the stimulation to growth that it receives from the testis hormone. Thirty-five days of additional underfeeding of the rats in *group* 65 (table 2) which had already been underfed for 58 days resulted in 25% greater loss of weight of the epididymis. Histologically, there was a conspicuous decrease in the number of formed elements in the epididymal duct. This coincided with the completion of the desquamatory stage in the testis tubules which occurs at this time.

Effect of chorionic gonadotropin on the accessory genitalia. The testes of the underfed rats increased in weight and activity in response to injections of CG and after 40 days of treatment, they weighed as much as those of the fully fed controls. On the other hand, the size, weight, and secretory activity of the accessory sex organs of the CG injected rats were greater than those of the normal rat. Stimulation of the accessory sex organs was due to the production of testosterone by the interstitial cells of the testes in response to CG. The increments of the weight of the prostate and seminal vesicle were due in part to cellular growth, and in greater part to the increase in the quantity of the prostatic and seminal fluids. The lower doses of CG resulted in a greater increase in the weight of the accessory genitalia than did larger doses. For example, doses of 10 R.U. elicited a continuous rise in the weight of the prostate. The gland enlarged from an average of 60 mg. before any injection to 630 mg. after 20 days and to 830 mg. after 44 days of treatment. The 15 R.U. dose given for 20 days caused the prostate to increase in weight to 350 mg., then to a maximum of 660 mg. on the 27th day, while after 44 days of treatment the prostate weight was 570 mg. The 25 R.U. dose resulted in a growth of the prostate to 600 mg. on the 20th day, to a

maximum weight of 740 mg. on the 23rd day, while on the 44th day it weighed 500 mg. The seminal vesicles and the rest of the genital tract showed changes in weight similar to those found to obtain for the prostate.

Effect of chorionic gonadotropin on the wheel-cells present in the interstitial tissue of the testis during underfeeding. In the normal rat, the nuclear chromatin of the Leydig cells is distributed evenly or is occasionally slightly clumped (fig. 2). During inanition, the clumping becomes very marked and the clumps are symmetrically arranged at the periphery of the nucleus, giving it a wheel-like appearance[2] (fig. 3).

Injections of CG (10 to 25 R.U.) into underfed rats, the left testes of which at biopsy showed the presence of wheel-cells, resulted in the disappearance of the wheel-cell formation in the remaining testis despite the continued undernutrition (fig. 4). The cells of the interstitial tissue and their nuclei were now much larger and the nuclear chromatin was more evenly distributed than in the normal or in the refed animals. A similar enlargement of the interstitial cells and of the nuclei occurs in the normal rat treated with CG. It is concluded that the interstitial cells of the undernourished testis respond to CG in a manner qualitatively similar to the normal.

Effect of chorionic gonadotropin on inanition sterility. Prolonged chronic undernutrition in the rat resulted in atrophy of the accessory sex organs and in aspermia. The rats were incapable of mating for these reasons. Experiments demonstrated that in the presence of continued undernutrition, injections of CG resulted in fertile matings. Such a result was to be expected from the fact that injections of CG gave histological evidence of spermatogenesis.

Four male rats underfed for 158 days and known to be sterile were chosen for this trial. The left testis and epididymis of each rat was removed surgically in order to determine the degree of genital atrophy incident to the underfeeding. On histological examination there were no spermatozoa in 3 of the epididymi and but a moderate number in the 4th. This latter rat was not injected with CG but kept as a control. Each of the 3 other underfed rats received 10 R.U. of CG per day except Sunday during the next 40 days or a total of 330 R.U. per rat. At the end of the 40 days, each of the 4 rats was placed overnight for several nights in a cage with a normal female rat. Positive matings occurred with 2 of the 3 rats which were being injected but not with the control. At subsequent autopsy, the 3 rats which had been injected with CG had hypertrophic accessory genital organs and numerous spermatozoa. On further trial, the control rat failed to mate successfully despite the fact that at autopsy the remaining testis and epididymis contained spermatozoa. The failure to mate was probably due to the fact that the accessory genital organs were markedly atrophic. An underfed rat which did not mate, but the biopsy testis of which contained spermatozoa, did mate successfully after treatment with the testosterone propionate.

SUMMARY

The depressant effects of acute starvation and of chronic underfeeding on the testes and the accessory genitalia of the male rat have been studied. It has been shown that these regressive changes were corrected by the injection of chorionic gonadotropin.

During chronic inanition the following were found.

(*a*) Atrophy of the accessory sex organs is primarily due to a diminution in testosterone production, because when small doses of chorionic gonadotropin were injected,

[2] These 'wheel-cell' formations occur in the ovarian stroma, and in the adrenal cortex of underfed rats (1). 'Wheel-cells' were first found by Selye et al. (16), in the atrophic ovaries of hypophysectomized rats. They attributed them as due to a hormonal deficiency resulting from removal of the pituitary gland.

which resulted in testosterone production, and when testosterone itself was administered (4, 14), the accessories increased in weight promptly and markedly.

(*b*) The atrophy of the interstitial cells of Leydig manifested itself by the appearance of a wheel-like formation in the nuclei of these cells. The appearance of 'wheel-cells' has been considered to be a reliable index of an insufficiency of gonadotropic hormone. The injection of chorionic gonadotropin resulted in the disappearance of the wheel-like formation in the nuclei of the interstitial cells.

(*c*) The loss in weight of the testes and the diminution in the size and spermatogenic activity of the testicular tubules were due to the lack of an adequate hormonal stimulus. The injection of chorionic gonadotropin resulted in growth of the testes and a return of spermatogenesis. The latter occurred only after 40 days of treatment, regardless of the dose (10–25 R.U.). The presence of an intact pituitary gland appears to be necessary for chorionic gonadotropin spermatogenesis in the underfed rat because chorionic gonadotropin cannot elicit spermatogenesis in the atrophic testes of hypophysectomized rats.[3]

(*d*) The sterility of the male rat is due to atrophy of the accessory genital organs and to a diminished or an absent spermatogenesis. Such sterile animals when injected with chorionic gonadotropin will both mate and also successfully impregnate normal female rats, despite the continued underfeeding.

It is concluded that in the chronically underfed male rat, the atrophy of the genital organs and the consequent sterility are due primarily to an insufficiency of gonadotropic hormones resulting from depression of the pituitary gland.

REFERENCES

1. Mulinos, M. G., and L. Pomerantz: *J. Nutr.* 19: 493. 1940.
2. Pomerantz, L., and M. G. Mulinos: *Am. J. Physiol.* 126: 601. 1939.
3. Mulinos, M. G., L. Pomerantz, J. Smelser and R. Kurzrok: *Proc. Soc. Exper. Biol. & Med.* 40: 79. 1939.
4. Mulinos, M. G., and L. Pomerantz: *Am. J. Physiol.* 132: 368. 1941.
5. Mason, K. E., and J. M. Wolfe: *Anat. Rec.* 45: 232. 1930.
6. Werner, S.: *Proc. Exper. Biol. & Med.* 41: 101. 1939.
7. Breneman, W. R.: *Endocrinology* 26: 1091. 1940.
8. Breneman, W. R.: *Endocrinology* 28: 946. 1941.
9. Stephens, D. J., and W. M. Allen: *Endocrinology* 28: 580. 1941.
10. Stephens, D. J.: *Journal of Clinical Endocrinology* 1: 257. 1941.
11. Jackson, C. M.: Inanition and Malnutrition. P. Blakiston's & Co., Philadelphia, 1925.
12. Siperstein, D. M.: *Anat. Rec.* 20: 355. 1921.
13. Mason, K. E.: *Am. J. Anat.* 52: 153. 1933.
14. Moore, C. R., and L. T. Samuels: *Am. J. Physiol.* 96: 278. 1931.
15. Smelser, G. K.: *Anat. Rec. Suppl.* 57: 28. 1933.
16. Selye, H., J. B. Collip and D. L. Thompson: *Endocrinology* 17: 494. 1933.

[3] We are indebted to Dr. Hans Selye of McGill University, Montreal, for furnishing us with a pregnancy urine extract prepared in his laboratory and which he reported to consist mainly of a substance stimulating the interstitial cells of the testis and having no effect on the atrophic germinal epithelium of hypophysectomized rats. With this substance we confirmed the effects produced by Follutein reported in the body of this paper.

EFFECTS OF HUMAN PREGNANCY SERUM ON PARTURITION IN THE RAT

SAMUEL S. ROSENFELD, BERNARD LAPAN AND HARRY BARON
From the Laboratories of Lebanon Hospital and The Jewish Memorial Hospital
NEW YORK CITY

A CAREFUL STUDY of the recent endocrinological literature reveals that very little work has been done on the serum of pregnant women, and none on the effect of this serum on pregnancy and parturition in experimental animals. Our present study concerns itself with this subject.

Before commencing work on this problem, a group of preliminary experiments was performed with non-pregnant animals. Sera obtained from men, non-pregnant women, and women in various stages of pregnancy, were injected subcutaneously into young mature female rats and rabbits. Injections of 0.1 to 0.5 cc. were given daily for a period of 14 days. The results obtained were briefly as follows.

Serum of	*Result*
Non-pregnant women	Mild to moderate stimulation of reproductive organs
Normal adult men	No stimulation of reproductive organs
Pregnant women (early)	Most marked stimulation of reproductive organs
Pregnant women (late)	Marked stimulation of reproductive organs

By stimulation is meant the enlargement of uterus and ovaries with hypertrophy of the myometrium, endometrial hyperplasia, and an increase in the number of ovarian follicles and corpora lutea.

MATERIALS AND METHODS

A series of 51 pregnant rats were used in this study. These rats have a gestation period of 21 to 22 days. Female rats whose vaginal smears showed an early estrous phase were impregnated by mating in separate cages, during the afternoon. The estrous phase was indicated by the presence of young epithelial cells and the absence of polynuclear cells and debris. The gestation period was calculated as starting on the day when spermatozoa were found in the vaginal washings, and pregnancy was confirmed by the presence of the placental sign (vaginal bleeding) on the 14th day of gestation. Daily injections of 0.25 to 1.0 cc. of serum were given the animals subcutaneously during the last third of the gestation period, and in some instances longer. The animals were observed daily during the period of injections. If delivery did not occur at term, the animals were autopsied in from 3 to 8 days. Where there was reason to suspect that pregnancy had been interrupted because of death of fetuses, laparotomy or autopsy before or at the due date was performed. Microscopic sections of organs were examined wherever necessary.

Nine pregnant rats were used as controls. Of these, 4 received no injections, 3 were injected with serum of non-pregnant women, and 2 with serum of normal adult men. Twenty-nine animals were injected with serum of pregnant women, some of whom were clinically abnormal (table 1). Ten more were injected with serum of women postabortal or postpartum. Three rats received serum and fluid from a patient with hydatid mole. The results are outlined in table 1.

RESULTS

The injection of serum of normal adult men and women, as controls, produced no demonstrable effect on the pregnant animals. Serum of pregnant women, whether

TABLE 1(a)

Rat No.	Serum used	Injection dates[1]	Total doses		Inhibition of parturition	Autopsy day[1]	Findings	
			Before term	After term			Ovaries	Fetuses
	Controls (No serum)							
	None	—	—	—	c	23	Moderate enlargement	Normal
	None	—	—	—	o	24	Corpora lutea	
	None	—	—	—	o	22		
	None	—	—	—	o	22		
	Controls (Serum)							
16	Non-preg. female 3 days premenstrual	14–20	2.5	o	o	22	Enlarged, corpora lutea	Normal
32	Non-preg. female 3 days postmenstrual	14–21	3.5	o	o	—	—	Normal
38	Non-preg. female 3 days postmenstrual	14–21	3.5	o	o	—	—	Normal
57	Normal male	15–21	3.0	o	o	—	—	Normal
94	Normal male	14–21	3.0	o	o	—	—	Normal
	Pregnancy serum							
2	Pooled normal preg.	14–21	3.0	o	+	24	Sl. enlarged, corpora lutea prominent	1 fetus alive, term size, 2 resorbed
18	Pooled normal preg.	14–22	2.4	0.4	+	25	1 ovarian cyst, numerous corpora lutea	Normal size
26	Normal preg. 6 mo.	15–22	3.0	0.5	+	24	Enlarged, corpora lutea Hem. follicles	Completely resorbed
72	Normal preg. 5 mo.	14–22	5.0	1.0	+	28	Numerous corpora lutea	Very large Resorbing
71	Normal preg. 8 mo.	17–20	2.5	o	+	22	Moderately enlarged Corpora lutea	Almost complete resorption
0	Habitual Abortion 4 mo. preg.	14	2.0	o	+	25	Hem. follicles	Resorbed
00	Habitual Abortion 4 mo. preg.	14	1.0	o	+	25	Numerous corpora lutea	Resorbing
1	Habitual Abortion 4 mo. preg.	15–25	2.0	1.5	+	28	Numerous corpora lutea	Resorbing
12	Habitual Abortion 4 mo. preg.	16–26	0.75	1.0	+	29	Markedly enlarged, corpora lutea	Resorbing
29	Habitual Abortion 4 mo. preg.	14–24	1.5	0.5	+	26	Enlarged, corpora lutea, Hem. foll.	Almost completely resorbed
58	Habitual Abortion 6 mo. preg.	16–22	2.0	o	o	—	—	Normal
9	Habitual Abortion 2 mo. preg.	14–26	3.5	2.0	+	28	Markedly enlarged	Resorbing
17	Threatened Abortion 8 wk. preg. & staining	16–17	1.5	o	+	21	Very markedly enlarged, corpora lutea & hem. foll.	Complete resorption
31	Threatened Abortion 8 wk. preg. and staining	14–20	1.5	o	+	23	Very markedly enlarged, corpora lutea & hem. foll.	Complete resorption
20	Threatened Abortion 8 wk. preg. & staining	14–17	0.5	o	o	—	—	Normal
44	Threatened Abortion 10 wk. preg. & staining	12–22	2.5	0.1	+	25	Markedly enlarged, corpora lutea, fol. cysts & hem. follicles	Resorbing
45	Threatened Abortion 10 wk. preg. & staining	12–22	2.4	0.1	+	30	Enlarged, corpora lutea & hem. foll.	Resorbing
7	Threatened Abortion 3 mo. preg. & staining	18–21	2.0	o	+	27	Markedly enlarged	Resorbed
14	Threatened Abortion 3 mo. preg. & staining	17–24	4.0	1.5	+	27	Markedly enlarged	Some alive, normal size others resorbing
19	Diabetes & eclampsia 2 mo. preg.	14–28	3.0	3.0	+	28	Markedly enlarged, corpora lutea	Complete resorption
37	Diabetes & eclampsia 7 mo. preg.	15–16	1.0	o	+	17	Slightly enlarged	Complete resorption
43	Eclampsia at term	15–22	3.0	0.5	+	28	Not unusual	Completely resorbed
23	Premature separation of placenta—fetus alive—6 mo. preg.	14–24	1.0	1.0	+	27	Enlarged, corpora lutea & hem. foll.	Almost complete resorption
40	Premature separation of placenta—fetus alive—6 mo. preg.	16–22	2.0	1.0	+	27	Not unusual	Comp. resorbed
46	Premature separation of placenta—fetus dead but undelivered	16–21	3.5	o	?	22	Prominent foll.	Four alive, others resorbing but very small
24	Hydatid mole serum before D & C	2–21	6.5	o	+	28	Tremendous ovaries, cysts & corpora lutea	Completely resorbed
28	Hydatid mole serum before D & C	14–24	3.5	1.5	+	26	Tremendous ovaries, cysts	Resorbing
49	Hydatid mole fluid	2–24	3.8	0.8	+	24	Very large	Resorbed

TABLE 1(a)—Continued

Rat No.	Serum used	Injection dates[1]	Total doses		Inhibition of parturition	Autopsy day[1]	Findings	
			Before term	After term			Ovaries	Fetuses
27	Immediately post-abortal	14–21	3.5	0	0	—	—	Normal
42	Immediately post-abortal 3 days	16–19	2.0	0	0	—	—	Normal
41	Immediately post-abortal 3 days	14–22	3.5	0.5	+	28	Not unusual	Completely resorbed
	Serum fractions							
73	Prolan precipitate	14–21	3.5	0	+	24	Moderately enlarged, corpora lutea	Resorbing
61	Filtrate (no prolan)	14–21	3.5	0	0	—	—	Normal
97	Prolan aged (no prolan)	14–20	2.0	0	0	—	—	Normal
78	Prolan inactivated	16–20	2.5	0	0	—	—	Normal
92	Active labor	14–20	2.5	1.5	+	26	Slightly enlarged, corpora lutea	Completely resorbed
7	36 hr. postpartum	14–22	3.0	0.5	+	25	Enlarged, corpora lutea	Resorbing, 1 larger than term size
	48 hr. postpartum	14–22	3.0	0.5	+	—	—	1 large macerated fetus delivered spont. with several placentae
91	60 hr. postpartum	15–21	3.5	0	0	—	—	—
1	3 days postpartum	16–19	3.0	0	0	—	—	Normal
91	9 days postpartum	14–19	3.0	0	0	—	—	Normal
96	9 days postpartum	15–21	4.0	0	0	—	—	Normal

[1] Numbers refer to the days of the gestation period of the rat.

normal or abnormal, at any stage and up to approximately 48 hours after delivery inhibited parturition or caused fetal death and subsequent resorption. One cc. of serum was the average minimum required to produce this effect. It was found that fetal resorption may proceed at various rates, not correlated with the amount of serum injected. In some instances, the abdomen of the animal and the size of the fetuses by palpation began to shrink rapidly within a day or two after injections were begun. In three instances resorption was complete at or before term, as shown by autopsy. The uteri in these cases were interesting in that the presence of fetuses was indicated only by small, yellowish, dense nodules, one to ten in number, projecting from the lateral margin of both uterine cornua (fig. 1). These measured 3 mm. to 5 mm. in diameter. The majority of animals which were overdue were autopsied 3 to 7 days after the due date. At this time the uterus was usually large, reddish-grey in color, and contained fetuses in varying degrees of autolysis and resorption. Although most of the fetuses were smaller than the normal fetus at term, in at least three in-

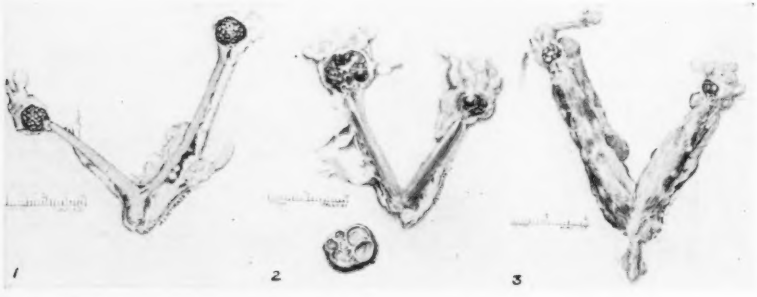


Fig. 1. UTERUS AND OVARIES SHOWING COMPLETE RESORPTION OF FETUSES AND OVARIAN STIMULATION. Three nodules in the right uterine horn indicate the recent presence of fetuses. Fig. 2. UTERUS AND OVARIES SHOWING COMPLETE RESORPTION OF FETUSES AND MARKED CYSTIC CHANGES IN THE OVARIES. Fig. 3. UTERUS CONTAINING RESORBING FETUSES. Nodules are forming laterally in both uterine cornua. Ovaries show moderate imcrease in size and number of corpora lutea.

stances, however, they were definitely larger. The uterus itself never showed evidence of infection or adhesions to surrounding viscera. The tone of the uterine muscle varied markedly. While in some animals there was strong contraction, leaving ring-like impressions on the fetuses, others showed relaxation. Only two instances of live postmature fetuses were encountered. In addition, there was one in which both living and resorbing fetuses were found when the animal was laparotomized on its due date.

The ovaries in practically all animals injected with pregnancy serum were larger than normal, and showed an increased number of corpora lutea. In seven instances hemorrhagic follicles were present, and in 2 animals there were found enormous follicular cysts (fig. 2). These findings were not encountered in the control animals.

Serum from which the gonadotropic factor had been removed by alcoholic precipitation or heating failed to delay parturition, whereas the crude gonadotropic fractions caused inhibition and resorption, with moderate stimulation of the ovary.

DISCUSSION

Normal pregnancy serum contains a factor or factors which are capable of inhibiting parturition in rats. It has been known for some time that pregnancy can be prolonged by a number of substances. A representative table of literature on this subject follows.

Authors	*Date*	*Animals Used*	*Substance Injected*	*Effect on Pregnancy and Labor*
Teel (1)	1926	Rat	A. P. fluid	Prolonged pregnancy 2–6 days
Evans & Simpson (2)	1929	Rat	Placental extr. PU extr.	Prolonged pregnancy
Allen & Corner (3)	1930	Rabbit	Progestin	Prolonged pregnancy or prevented labor
Nelson, Pfiffner & Haterius (4)	1930	Rat	Progestin	Prolonged pregnancy 2 days
Levin, Katzman & Doisy (5)	1931	Rat	PU extract	Prolonged pregnancy or prevented labor
Mandelstamm & Chaykowskiy (6)	1932	Mouse	Progestin	Prolonged pregnancy 4–10 days
Hain (7)	1932	Rat	A. P. extr. A. P. implant PU extr. A. P. growth hormone	Prolonged pregnancy 4–10 days
Courrier & Kehl (8)	1933	Rabbit	Progestin	Prevented labor
Snyder (9)	1934	Rabbit	PU extr.	Prolonged pregnancy
Hoopes (10)	1934	Rat	PU extr.	Prolonged pregnancy
Hoopes & King (11)	1935	Rat	PU extr.	Prolonged pregnancy
Watts (12)	1935	Rat	A. P. extr.	Prolonged pregnancy 2–5 days
Hain (13)	1935	Rat	Estrogen	Prolonged pregnancy 3–5 days
Koff & Davis (14)	1937	Rabbit	PU extr. Progesterone	Prolonged pregnancy
Heckel & Allen (15)	1937	Rabbit	Progesterone	Prolonged pregnancy
Hamilton & Wolfe (16)	1938	Rat	Testosterone	Prolonged pregnancy
Heckel & Allen (17)	1938	Rabbit	Synthetic estrogen	Prolonged pregnancy or prevented labor
King (18)	1938	Rat	PU extr.	Prolonged pregnancy

A review of the foregoing table shows that the prolongation of pregnancy or inhibition of labor can be produced by gonadotropins, including anterior pituitary extracts and pregnancy urine extracts, as well as by progestin, estrogen and testosterone. The substances most apt to be responsible for the inhibition of parturition in our experiments are the gonadotropins present in the serum injected. This assump-

tion is based on the fact that the animals injected with sera from which the gonadotropin was removed gave birth on time, whereas the same sera before fractionation caused prolongation of pregnancy. We found that this substance leaves the circulation within 72 hours after birth or abortion.

The explanation usually given for this parturition inhibiting action of gonadotropin is that new corpora lutea are produced in the ovaries, and that the resulting increased secretion of progestin causes uterine relaxation. Reynolds (19) believes that there is also a direct relaxing effect of the gonadotropins on the myometrium which precedes the development of the secondary ovarian effect. He further states that the gonadotropins may also produce initial contraction of the uterus which can cause fetal death by pressure. This may explain the occurrence of premature death of fetuses in utero in our experiments.

Our average normal and pooled pregnancy sera assayed only 1 R.U. of estrogen per cc. and 3 to 10 R.U. of gonadotropin per cc. Thus a total of no more than 50 R.U. of gonadotropin was used in any one of our experiments and yet our results were similar to those of others who employed hundreds of units of commercial preparations of pregnancy urine extracts. Furthermore, in our experiments we have noted more frequent death of fetuses than has been reported by workers using pregnancy urine extracts and pituitary gland extracts. We found a greater frequency of early death of fetuses in utero, and a very low incidence of live postmature fetuses. It would seem to us that the reason for this potency resides in the fact that the hormones in pregnancy serum may be more effectively combined, acting synergistically, or that there is an actual qualitative difference. This would be in accord with the work of Boycott and Rowlands (20), namely that human pregnancy serum produces much greater effects in non-pregnant animals than comparable amounts of pregnancy urine extracts.

SUMMARY

Human pregnancy serum, when injected into pregnant rats, inhibits parturition and causes early death of fetuses and their resorption. It also produces marked stimulation of the ovaries, with formation of additional corpora lutea, follicular cysts and occasional hemorrhagic follicles. These effects are most probably caused by the gonadotropins present in the serum, and disappear within 72 hours following delivery or abortion.

The gonadotropins in human pregnancy serum appear to differ quantitatively and possibly qualitatively from those in pregnancy urine extracts and pituitary gland extracts.

REFERENCES

1. Teel, H. M.: *Am. J. Physiol.* 79: 170. 1926.
2. Evans, H. M., and M. E. Simpson: *Proc. Soc. Exper. Biol. & Med.* 26: 595. 1929.
3. Allen, E., and G. W. Corner: *Proc. Soc. Exper. Biol. & Med.* 27: 403. 1930.
4. Nelson, W. O., J. J. Pfiffner and H. O. Haterius: *Am. J. Physiol.* 91: 690. 1930.
5. Levin, L., P. A. Katzman and E. A. Doisy: *Endocrinology* 15: 207. 1931.
6. Mandelstamm, A., and V. K. Chaykowskiy: *Zentralbl. f. Gynäk.* 56: 2346. 1932.
7. Hain, A. M.: *Quart. J. Exper. Physiol.* 22: 249. 1932.
8. Courrier, R., and R. Kehl: *Compt. Rend. Soc. de Biol.* 114: 1317. 1933.
9. Snyder, F. F.: *Bull. Johns Hopkins Hosp.* 54: 1. 1934.
10. Hoopes, E. C.: *Proc. Soc. Exper. Biol. & Med.* 31: 1115. 1934.
11. Hoopes, E. C., and J. L. King: *Am. J. Physiol.* 111: 507. 1935.
12. Watts, R. M.: *Am. J. Obst. & Gynec.* 30: 174. 1935.
13. Hain, A. M.: *Quart. J. Exper. Physiol.* 25: 131. 1935.
14. Koff, A. K., and M. E. Davis: *Am. J. Obst. & Gynec.* 34: 26. 1937.
15. Heckel, G. P., and W. M. Allen: *Am. J. Physiol.* 119: 330. 1937.
16. Hamilton, J. B., and J. M. Wolfe: *Anat. Rec.* 70: 433. 1938.
17. Heckel, G. P., and W. M. Allen: *Am. J. Physiol.* 123: 95. 1938.
18. King, J. L.: *Am. J. Physiol.* 122: 455. 1938.
19. Reynolds, S. R. M.: *Am. J. Physiol.* 100: 545. 1932.
20. Boycott, M., and J. W. Rowlands: *Brit. M. J.* 1: 1097. 1938.

THE QUESTION OF AN AQUEOUS HORMONE FROM THE TESTICLE[1]

DAVID RUBIN

From the Hull Zoological Laboratory, The University of Chicago

CHICAGO, ILLINOIS

MOTTRAM AND CRAMER (1) appear to have been the first to suggest, on the basis of experimental findings, that the generative tissue of the testis secretes a substance distinct and independent from the hormone presumed to come exclusively from the interstitial cells of Leydig. Support for this notion has come from the investigations of Martins and Rocha (2), Myers and co-workers (3, 4), McCullagh and Walsh (5), and Vidgoff and associates (6, 7), as well as others. These investigations have led the above workers to concur in the idea that the germinal epithelium secretes an aqueous hormone (inhibin) that exercises the known inhibitory action of the testicle on the pituitary gland without directly influencing the accessory reproductive glands and, second, that the lipoid hormone contributed by the Leydig cells and controlling the accessory reproductive glands is without effect on the pituitary. Thus, the testicle would produce a dual secretion rather than the one hormone generally credited with the regulation of both of these activities.

In view of the lack of evidence to confirm certain logical phases of this concept (8, 9, 10) Professor Carl R. Moore suggested that the author conduct a series of experiments to test the effects of orally administered desiccated testis material, and the effects of injections of testicular aqueous extracts upon the testes and accessory sex glands of the albino rat.

This account deals with the experiences and findings during the course of the investigation and agrees in casting doubt on the contentions regarding such an aqueous hormone.[2]

MATERIALS AND METHODS

Animals employed in this study were selected from the thriving colony maintained in this laboratory. In each of the 3 series to be described the litter has been used as a unit, that is, treated animals were always compared with untreated littermates.

The desiccated material was prepared from fresh bull testes, ground, spread on flat trays, and placed in a drying oven at 60 to 70° C. The yield was approximately 10% of the original substance.

Aqueous extracts of bull testes and hog liver were prepared by grinding the fresh tissues, precipitating the proteins with anhydrous sodium sulfate at 32° C., straining the resultant mash through a fine cloth, and then filtering the crude extract through a Büchner funnel. The filtrate was then placed in the ice box at 0° C. and the Na_2SO_4

[1] This investigation has been aided by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

[2] It is a pleasure to acknowledge the ready cooperation and generous advice of Professor Carl R. Moore throughout the course of the investigation. I am also indebted to Professor S. Wright for his helpful advice in the analysis of the data, to Professor W. C. Allee for placing the facilities of the Whitman Laboratory at my disposal, and, to Dr. D. Price for confirming the studies of prostate gland histology.

allowed to crystallize out. The protein-free fluid was treated with benzene and the fatty fraction discarded. The clear protein-free, fat-free extract was concentrated so that 1 cc. of the final aqueous solution was equivalent to 25 gm. of the original mash and contained 4% Na_2SO_4. A more detailed account of this procedure will be found elsewhere (6).

Animals were killed by decapitation on the day following the last treatment. Testes and accessory reproductive organs were removed, weighed immediately on a torsion balance, and fixed in Bouin's fluid.

The tissues were sectioned at 6 to 7μ. Testes and seminal vesicles were stained with Erlich's hematoxylin, the ventral prostates with Harris' hematoxylin.

RESULTS

A. *Testis feeding.* The testicular material described above was administered to males of 5 litters and to females of 3 litters of albino rats. Gelatin capsules containing 0.2 to 1.2 gm. of the desiccated substance were employed. Fed animals were individually caged.

Males. Minimum doses of 0.2 gm administered daily for 79 days and a maximum of 1.2 gm. daily for 114 days (see table 1 for details) failed to produce any changes in the testes or accessory sex glands of reproduction. The seminal vesicles, one of the

TABLE 1. SERIES I. COMPARISON OF THE ARITHMETIC MEANS OF BODY AND PAIRED ORGAN WEIGHTS OF MALE ALBINO RATS FED POWDERED, DESICCATED BULL TESTIS AND OF NORMAL LITTER-MATE CONTROLS

Litter	No. animals	Treatment		Autopsy		Testes	Seminal vesicles	Ventral prostate	Cowper's glands
		gm./day	No. days	Age	Weight				
				days	gm.	gm.	gm.	gm.	gm.
A	2[1]	—	—	138	263	2.74	0.7304	0.2743	0.0897
	2	0.2	79		262	2.70	0.7222	0.2965	0.0770
B	2[1]	—	—	194	364	3.00	1.2032	0.4650	0.1009
	3	0.2	119		332	2.97	1.0471	0.3630	0.1390
C	3[1]	—	—	158	315	3.05	1.1481	0.3837	0.0768
	3	0.4	122		315	2.85	1.0273	0.3392	0.0839
D	2[1]	—	—	156	350	2.92	1.1992	0.3915	0.1254
	2	1.2	114		335	3.22	1.0873	0.4282	0.1028
E	2[1]	—	—	161	326	2.70	1.0207	0.4605	0.1025
	2	0.6	114		300	2.83	1.1231	0.5560	0.1181

[1] Normal untreated controls.

most sensitive indicators of diminished male hormone in the rat, showed no signs of atrophy. Animals receiving the highest total dosage had slightly heavier testes and ventral prostates than the corresponding untreated litter-mate controls (table 1). Motile spermatozoa were found in the epididymides of all animals.

Histologically the testes of the treated rats revealed complete stages in spermatogenesis. Light areas and secretion granules were clearly defined in the ventral prostates and seminal vesicles, respectively, of both experimental and control animals.

Females. Daily administration of 0.2, 0.4 and 0.6 gm. of desiccated testis for 125, 116 and 125 days, respectively, to 3 groups of female rats failed to prolong or inhibit estrous cycles. This was established by taking vaginal smears for 3 weeks subsequent to approximately 9 weeks of testis feeding.

At autopsy only normal variations in ovarian weights were noted. The largest ovaries, 0.1002 gm., were found in an animal receiving a daily dose of 0.4 gm. for 125 days.

B. Injections of testicular aqueous extracts and Na_2SO_4. The aqueous extract of bull testes or a 4% Na_2SO_4 solution[3] was injected subcutaneously in daily doses of 1 cc. amounts for periods of 20 and 21 days into young, sexually mature, male animals (85–115 days) of 4 litters. Litter-mates served as untreated controls (table 2).

At autopsy the sex accessories of the injected animals were found to be lighter in weight than the control glands. This decrease while more apparent in the testis extract recipients was also detectable in the injected control rats. Other comparisons revealed that body weight increments observed in the untreated control rats (except *litter A1*) during the experimental period were lacking in the testis extract injected

TABLE 2. SERIES II. COMPARISON OF THE ARITHMETIC MEANS OF BODY AND PAIRED ORGAN WEIGHTS OF T.E.I.,[1] Na_2SO_4[2] INJECTED AND RESTING CONTROL LITTER-MATE MALE ALBINO RATS

Litter	No. animals	Treatment	Initial weight	Autopsy		Testes	Seminal vesicles	Ventral prostate	Cowper's glands
				Age	Weight				
			gm.	*days*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
	2	Normal control	285		274	2.81	1.1583	0.3579	0.0862
A1	2	Na_2SO_4	246	135	245	2.68	0.8865	0.2888	0.0867
	2	T.E.I.	265		219	2.48	0.2880	0.2231	0.0625
	2	Normal control	232		264	2.81	0.8321	0.3350	0.0740
B1	2	Na_2SO_4	186	119	210	2.59	0.8209	0.3119	0.0641
	2	T.E.I.	208		208	2.55	0.5923	0.2200	0.0540
	2	Normal control	198		242	3.15	0.9857	0.2623	0.0833
C1	2	Na_2SO_4	186	105	221	2.69	0.7816	0.2326	0.0650
	3	T.E.I.	191		185	2.50	0.3078	0.1816	0.0469
	2	Normal control	193		215	2.60	0.8597	0.2629	0.0673
D1	2	Na_2SO_4	168	111	200	2.31	0.6893	0.2830	0.0619
	2	T.E.I.	189		168	2.36	0.2029	0.1538	0.0419

[1] Testicular extract (aqueous) injected, 1 cc. administered subcutaneously each day for 20 days in litters *A1*, *B1* and *C1*, and for 21 days in litter *D1*.

[2] 4% solution of sodium sulfate, 1 cc. administered subcutaneously each day for 20 days in litters *A1*, *B1* and *C1*, and for 21 days in litter *D1*.

groups and but slightly expressed in the Na_2SO_4 injected groups. In fact, the animals receiving testis extract injections suffered fairly pronounced weight losses in 3 of the 4 litters. Of even greater significance was the reduction in accessory sex gland weights which paralleled fairly closely the diminished body weight following such treatment.

Histological studies disclosed the presence of secretion granules in the seminal vesicles and conspicuous light areas in the prostate glands of both treated and untreated animals. No marked epithelial reduction was detected. The testes of treated animals were normal in every respect.

These findings raised two questions. (*A*) Are the weight changes in the seminal vesicles and prostate gland due to a diminished testis hormone secretion? And, (*B*) if so, is there a specific principle in the testis extract to which this condition is attributable, or is there some other factor involved, one which might perhaps be linked with the observed body weight losses? That the latter possibility was not too untenable was suggested by the similar, although less obvious, trend obtained with Na_2SO_4 injections.

[3] A control of the Na_2SO_4 content in the extract (see procedure under Materials and Methods).

In order to cast some light on this problem the following series was designed to determine whether the same or similar effects would be elicited with an identically prepared extract of a glandular organ other than testis.

C. *Injections of testicular and liver aqueous extracts.* Six litters of male rats were used in this series. Two or more animals of each litter (table 3) received daily subcutaneous injections of the aqueous extracts of liver or testis in 1 cc. doses for a period of 28 days. At autopsy they provided treated and control material, ranging in age from 107 to 151 days.

TABLE 3. SERIES III. COMPARISON OF THE ARITHMETIC MEANS OF BODY AND PAIRED ORGAN WEIGHTS OF T.E.I.,[1] L.E.I.[2] AND NORMAL CONTROL LITTER-MATE MALE ALBINO RATS

Litter	No. animals	Treatment	Initial weight	Autopsy		Testes	Seminal vesicles	Ventral prostate	Cowper's glands
				Age	Weight				
			gm.	days	gm.	gm.	gm.	gm.	gm.
	2	Normal control	269		282	2.75	1.0833	0.3316	0.0899
A2	2	L.E.I. control	242	151	242	2.35	1.0148	0.2827	0.0811
	2	T.E.I.	247		234	2.46	0.8832	0.2274	0.0719
	2	Normal control	278		291	2.74	1.0530	0.3565	0.0884
B2	2	L.E.I. control	263	150	243	2.53	0.8075	0.2980	0.0887
	2	T.E.I.	265		241	2.68	0.7350	0.3068	0.0828
	2	Normal control	243		252	3.00	1.0456	0.3414	0.1037
C2	2	L.E.I. control	212	119	236	2.70	0.8647	0.2551	0.0858
	2	T.E.I.	238		237	2.93	0.8989	0.2597	0.0829
	2	Normal control	194		229	2.67	0.9407	0.2216	0.0601
D2	3	L.E.I. control	219	118	215	2.49	0.8455	0.2343	0.0619
	3	T.E.I.	228		210	2.70	0.8174	0.1753	0.0591
	3	Normal control	233		255	2.78	0.8755	0.2543	0.0715
E2	2	L.E.I. control	239	119	243	2.69	0.7139	0.2916	0.0642
	2	T.E.I.	250		228	2.78	0.7472	0.1877	0.0664
	2	Normal control	202		235	2.54	0.7722	0.2658	0.0710
F2	2	L.E.I. control	201	107	231	2.75	0.7929	0.2497	0.0795
	2	T.E.I.	191		214	2.79	0.6586	0.2317	0.0762

[1] Testicular extract (aqueous) injected animals, 1 cc. daily for 28 days.
[2] Liver extract (aqueous) injected animals, 1 cc. daily for 28 days.

Body weight reductions varying from 4 to 9% of the initial weight (mean comparisons) were found in testis extract recipients of 5 litters. The liver extract-injected animals of litters *B2* and *D2* (table 3) showed mean body weight decreases of 7.9, and 1.9%, respectively, while in a third litter, *A2* the initial weight was maintained. Untreated control animals gained uniformly.

The testes appeared unaffected by treatment. Weight comparisons revealed little difference between the testicles of any of the 3 groups under consideration. Complete stages in spermatogenesis were readily detectable.

Slight decreases in the average weight of the seminal vesicles and ventral prostates of the treated animals were noted. In this as in the preceding series it is significant that in most instances the animals exhibiting the greatest weight losses also exhibited the lightest accessory sex glands (table 3). Atrophic changes such as described by other investigators were not observed. Upon examination the above organs were found to be normal in all respects. Numerous secretion granules were seen in the epithelial cells of the seminal vesicles, light areas were clearly defined in the ventral prostates, and both glands possessed an abundance of colloid in all cases.

Careful study of the absolute mean values in table 3 discloses the unmistakable parallelism in the degree of body and accessory sex gland weight reduction when well controlled series of rats are treated with aqueous extracts of liver and testis.

Probability values (table 3a), obtained by subjecting the data to Student's method of paired comparisons of the means, corroborates this view. The two exceptions, one in the prostate comparison between normal control and liver extract-injected animals and the other in the testis comparison between the two extract-injected groups (table 3a), can be satisfactorily explained in terms of the small samples used in this analysis. Concerning the ventral prostate inconsistency ($P=0.2$) two factors must be taken account of: that no significance was expressed between both extract-injected groups ($P=0.1$), and that comparisons of the seminal vesicles, the most sensitive and therefore the most reliable indicators, are entirely consistent with the interpretation of the observed effects. In the case of the testis comparison ($P=0.01$) it must be noted that the standard error is unusually small (approximately 0.3 of the standard error in the other testis comparisons). This may be attributed to a chance phenomenon whereby the mean differences all tended in the same direction, although each individual mean difference was less than the maximum mean difference observed in the other comparisons. This fact can be readily established from the absolute values given in table 3.

DISCUSSION

For the purpose of facilitating the discussion a recapitulation of the salient features of this investigation may be listed as follows: (*a*) desiccated testicular material fails to produce any changes in the reproductive tract of either male or female albino rats; (*b*) accessory sex gland weight depression obtained with testicular aqueous extracts is also obtained with liver aqueous extracts and to a lesser degree with Na_2SO_4 injections; (*c*) such reductions seem to parallel the total body weight decreases resulting from the use of these substances; (*d*) weight decreases in the seminal vesicles and prostate gland are the only evidence of a possible impairment of the endocrine function of the testis, for histologically no sign of castration atrophy is discernible; (*e*) the gross size, weight and spermatogenic activity of the testis remains unaffected by any of the treatments described.

TABLE 3a. SUMS OF THE DIFFERENCES OF THE MEANS (Δ), THEIR STANDARD ERRORS, AND PROBABILITY VALUES (P) FOR BODY AND SEX ORGAN WEIGHTS. (P=0.05 OR LESS INDICATES SIGNIFICANCE)

Comparisons[1]	Body weight				Testes		Seminal vesicles		Ventral prostate	
	Initial		Autopsy							
	Δ	P	Δ	P	Δ	P	Δ	P	Δ	P
	gm.		gm.		gm.		gm.		gm.	
N.C.-L.E.I.	43±8.7	0.5	134±7.36	0.02	0.97±.086	0.1	0.73±.089	0.02	0.38±.011	0.2
N.C.-T.E.I.	0±0.0	1.0	180±6.22	0.01	0.14±.071	0.8	1.03±.032	0.01	0.22±.018	0.01
L.E.I.-T.E.I.	−43±4.7	0.2	46±2.92	0.05	−0.83±.030	0.01	0.30±.031	0.2	0.16±.019	0.1

[1] N.C.=Normal control; L.E.I.=Liver extract injected; and T.E.I.=Testis extract injected.

The hypothesis that a specific pituitary-inhibiting, water-soluble hormone is resident in a testicular aqueous extract appears untenable in the light of these findings. On the other hand a more suitable and perhaps a more nearly correct interpretation is made possible by reference to the work of Moore and Samuels (11). Briefly stated, it was found that the hormone output capacity of the rat testis is influenced by the nutritive state of the organism. Animals suffering body weight losses, as a result of partial inanition or vitamin B deficiency diets, exhibited seminal vesicle and

prostate gland changes in parallel with such losses. A significant feature of this phenomenon is that despite accessory sex gland weight decreases the gross size and gametogenic activity of the testis remained normal. Other phases of this problem led the above investigators to advance the theory that a poor nutritive state in the organism effects the pituitary gland, thus lowering the amount of sex-stimulating hormone and indirectly interfering with the endocrine function of the testis. This aspect of the work was clinched by the reports of Mason and Wolfe (12), and Evans and Simpson (13).

The striking resemblance between the findings of Moore and Samuels and those obtained in this study is highly suggestive of a similarly directed action in both cases. It would seem that in the absence of a very low nutritive state, but nonetheless a recognizable one, the hormone output of the testis is affected only sufficiently to depress slightly the weights of the seminal vesicles and prostate gland; still greater depression would be required before influencing the histological character of these organs. That the effects observed in this investigation are probably of this nature is further attested to by the parallelism in response elicited with testicular aqueous and liver aqueous extracts.

It would thus appear that whereas a number of results have been obtained which seem to involve the germinal epithelium in the hormone production of the testicle (1, 14–17) the observations in the course of this investigation do not support those findings, advanced as proof of this involvement, that are based on the oral administration of desiccated testicular material and the injection of aqueous extracts of bull testis (3–7).

The finding that desiccated testicular material administered to female rats is entirely ineffective in modifying the normal estrous cycle is also at variance with the claims of several of the proponents of an aqueous hormone. It is of interest, in this respect, to take note of the fact that inhibition of the estrous cycle can be achieved with androgen injections (18, 19).

While it has been demonstrated that desiccated testicular substance and testicular aqueous extracts do not contain a specific pituitary inhibiting principle, the question of a germinal epithelium secretion must admittedly remain open. Recognition is, however, to be taken of the fact that a number of workers (8, 9, 10, 20, 21, 22) have shown that a single testis hormone is capable of exercising the two functions, namely pituitary inhibition and accessory sex gland maintenance, which some contend are separable phenomena controlled by two distinct testicular secretions.

SUMMARY

Effects of desiccated testicular feeding. Prolonged daily feeding of desiccated bull testis to young, sexually mature, male albino rats was found to have no effect, gross or histological, on either the testis or the accessory sex glands.

The same substance, when fed for similar periods of time to adult female albino rats, failed to inhibit or in any way modify the estrous cycles.

Effects of an aqueous extract of bull testis. (*a*) *Series I.* Young, sexually mature, male albino rats treated with an aqueous extract of bull testis exhibited body weight and accessory sex gland weight reductions. A control solution of Na_2SO_4 effected similar but less conspicuous changes. The testes remained normal in both groups of treated animals. Histological studies failed to reveal any structural modifications.

(*b*) *Series II.* Liver and testicular aqueous extracts produced essentially the same degree of reduction in body and accessory sex gland weights. In most instances the weight reductions in the latter structures paralleled the body weight losses. Histologically the accessories and the testes appeared to be normal.

The experimental evidence indicates that (*a*) neither desiccated testis material nor testicular aqueous extracts contain a specific pituitary-inhibiting principle; (*b*) any changes in the reproductive structures produced by injecting aqueous extracts of liver or testis are probably due to an indirect effect as a result of a change in the nutritive state of the animal.

More research is needed to establish the role of the germinal epithelium in the production of the male sex hormone.

REFERENCES

1. Mottram, J. C., and W. Cramer: *Quart. J. Exper. Physiol.* 13: 209. 1923.
2. Martins, T., and A. Rocha: *Endocrinology* 15: 421. 1931.
3. Myers, H. B., B. Vidgoff and W. C. Hunter: *Proc. Soc. Exper. Biol. & Med.* 30: 1099. 1933.
4. Myers, H. B., B. Vidgoff, G. H. Schade and W. C. Hunter: *Endocrinology* 20: 389. 1936.
5. McCullagh, D. R., and E. L. Walsh: *Endocrinology* 19: 466. 1935.
6. Vidgoff, B., R. Hill, H. Vehrs and R. Kubin: *Endocrinology* 25: 391. 1939.
7. Vidgoff, B., H. Vehrs and R. Hill: *West. J. Surg. Obst. & Gynec.* 46: 648. 1938.
8. Moore, C. R., and D. Price: *Am. J. Anat.* 50: 13. 1932.
9. Nelson, W. O., and T. F. Gallagher: *Anat. Rec.* 64: 129. 1935.
10. Allanson, M.: *Proc. Roy. Soc. B.* 124: 196. 1937.
11. Moore, C. R., and L. T. Samuels: *Am. J. Physiol.* 96: 278. 1931.
12. Mason, K. E., and J. M. Wolfe: *Anat. Rec.* 45: 232. 1930.
13. Evans, H. M., and M. E. Simpson: *Anat. Rec.* 45: 216. 1930.
14. Evans, H. M., and M. E. Simpson: *Am. J. Physiol.* 89: 371. 1929.
15. Korenchevsky, V.: *J. Path. & Bact.* 33: 607. 1930.
16. Moore, C. R.: *Cold Spring Harbor Symp. Quant. Biol.* 5: 117. 1937.
17. Hanes, F. M., and C. W. Hooker: *Proc. Soc. Exper. Biol. & Med.* 35: 549. 1937.
18. Ihrke, I. A., and F. D'Amour: *Am. J. Physiol.* 96: 289. 1931.
19. Browman, L. G.: *Proc. Soc. Exper. Biol. & Med.* 36: 205. 1937.
20. Moore, C. R., and D. Price: *Endocrinology* 21: 313. 1937.
21. Reese, J. D., and M. McQueen-Williams: *Am. J. Physiol.* 101: 239. 1932.
22. Nelson, W. O.: *Proc. Soc. Exper. Biol. & Med.* 31: 1192. 1934.

NOTES AND COMMENTS

WILL ANTERIOR PITUITARY-LIKE SUBSTANCE PREVENT THE DEGENERATIVE CHANGES IN THE TESTIS INCIDENT TO CRYPTORCHIDISM?

IT IS GENERALLY AGREED that the degenerative changes in the undescended testis are due to its abnormal position, although occasionally such testes may be congenitally malformed. An aberrant position is detrimental to a scrotal testis, because of the harmful effects of the increased body temperature. An abdominal testis is not always incompatible with spermatogenesis, however, as in certain species, as the elephant, rhinoceros and in birds, the testes are normally intra-abdominal. For this and other reasons the hormonal effects on the development of the testes are considered to be important.

In cryptorchidism, the interstitial tissue is relatively increased in amount and there is degeneration of the tubules, with lack of differentiation of the spermatogenic elements. The value of some means other than operation by which this degeneration of the spermatogenic cells of the tubules could be prevented is apparent.

The interrelationship of spermatogenesis and function of the pituitary gland is well known. Anterior pituitary-like substance has been used in the treatment of cryptorchidism. Its use began following Engle's observation[1] that precocious descent of the testis could be produced in the macaque monkey by the injection of gonadotropic substances. The rationale for its use otherwise has little basis and the dosages and methods of administration are empirical. While the early reports of descent following anterior pituitary-like substance were spectacular, later reports were less encouraging.[2]

Moore and Price[3] have shown that androgenic substances from bull testis, when injected into males are harmful to the testis rather than beneficial. The injurious effects are proportional to the dosage and immaturity of the animal. However, in the ground squirrel, androsterone causes the appearance of spermatozoa at periods of the year earlier than normal. From a review of the available literature, the effect of androsterone and pituitary-like substances on the histology of the undescended testes have not been ascertained, except for the report by Eisenstaedt, Appel and Frankel.[4] These authors believe that hormonal therapy may cause degeneration of the cryptorchid gland. The number of animals used in their experimental study was not large, however, and it is difficult to evaluate clinical results if there is no control study.

The purpose of these experiments is to determine whether anterior pituitary-like and androgenic substance may prevent the degenerative changes incident to cryptorchidism.

Method. Young and old male rats and guinea pigs were used as the experimental animals. Under ether anesthesia, the right testis was elevated to the abdomen and the inguinal canal closed by silk sutures. Some animals were used as controls, while others were given subcutaneous injections of anterior pituitary-like substance or testosterone propionate. At the end of various lengths of time, the animals were killed. The testes were fixed in 10% formalin,

[1] ENGLE, E. T.: in Sex and Internal Secretions, Williams & Wilkins, Baltimore, 1932.

[2] THOMPSON, W. D., A. D. BEVON, U. J. HECKEL, E. R. MCCARTHY AND P. K. THOMPSON: *Endocrinology* 21: 20. 1937, MIMPRISS, T. W.: *Lancet* 1: 497. 1937, REA, C. E.: *Arch. Surg.* 38: 1054. 1939.

[3] MOORE, C. R., AND D. PRICE: in Sex and Internal Secretions, Williams & Wilkins, Baltimore, 1932.

[4] EISENSTAEDT, J. S., M. APPEL AND M. FRAENKEL: *J.A.M.A.* 115: 200. 1940.

sectioned and stained with hematoxylin-eosin. An evaluation of the effects of the hormonal substance on the histology of the undescended testis was made.

A. *The effect of APL on the cryptorchid testis of the rat.* (*a*) *Young rat.* Young rats about 2 months old were used in these experiments. The right testicle was rendered cryptorchid and one week later, subcutaneous injections of APL (Ayerst, McKenna & Harrison), 100 rat units (1 cc.), were given every other day for a total of 2500 U. Four weeks later the animals were killed and the histology of the treated and untreated cryptorchid gonads studied.

Of 10 young rats so treated and 6 animals receiving no injections (controls), the undescended testes showed the usual degenerative changes incident to cryptorchidism. There was no difference between the treated and control group of animals. There was cytolysis of the germinal epithelium and the tubular lumina were filled with debris. No mature germinal cells were seen. Some of the degeneration in the ectopic testis was probably due to the operative manipulation, because the testes of two untreated animals that died within 10 days after operation showed degrees of degeneration of the germinal epithelium.

(*b*) *Adult rats.* Similar experiments were carried out on adult rats. The APL failed to protect the retained testis against the usual degenerative changes which were just as marked in the untreated ectopic testis. The degree of degeneration in the gonads in the older rats seemed to be less than in those of the younger animals.

The dose of APL, while excessive for the rat, was in itself probably not deleterious as no difference in the amount of degeneration could be seen between the treated and untreated undescended testes.

(*c*) *Effect of increasing the interval between operation and injection of APL on cryptorchid testes of old rats.* In 4 adult rats, injections of APL were not begun until 2 months after operation. The APL did not lessen the degree of degenerative changes in the treated as compared to those in two untreated animals. In fact, the degenerative changes were quite marked and irreparable in both series.

B. *The effect of APL on the cryptorchid testis of the guinea pig.* (*a*) *Young guinea pigs.* Twelve young guinea pigs about 3 months old were used in this series. Eight with a right cryptorchidism were treated with APL subcutaneously, 100 R.U. (1 cc.), every other day until 2500 U were given. Four other guinea pigs with a unilateral, experimentally produced cryptorchidism were used as controls. One month after operation, both the treated and untreated animals were killed and the testes examined microscopically. No differences were discernible between the treated and untreated cryptorchids. The germinal epithelium had lost its typical arrangement. Cytolysis and fragmentation within the tubules were marked. The lumina contained debris but no mature germinal cells.

(*b*) *Adult guinea pigs.* The cryptorchid testes of 6 adult guinea pigs treated similarly to those described in (*a*) showed as much degeneration as those of 4 untreated ectopic gonads.

C. *Effect of testosterone propionate on the cryptorchid testis.* (*a*) *The Rat.* In 6 adult rats, the right testis was rendered cryptorchid and the animal then given 10 mg. of testosterone proprionate (Schering), 3 times a week for 4 weeks. Four other rats with unilateral cryptorchidism were used as controls. At the end of 4 weeks, histologic sections failed to show any prevention of the degenerative changes incident to cryptorchidism in the treated cases as compared to those of the untreated cases.

(*b*) *The guinea pig.* In 4 adult guinea pigs with unilateral cryptorchidism treated with testosterone propionate by the same method as in (*a*) no protection against degeneration was noted when the histologic sections were compared to the cryptorchid testes of 2 untreated guinea pigs.

Summary. APL or testosterone propionate in the doses and methods used in these experiments did not prevent the degenerative changes incident to cryptorchidism in either young or adult rats or guinea pigs. In these experiments in contrast to the work of Eisenstaedt, Appel and Frankel, APL did not seem to increase the degenerative changes in the cryptorchid testis.

CHARLES E. REA

Department of Surgery
University of Minnesota Medical School
MINNEAPOLIS, MINNESOTA

INACTIVATION OF ESTROGENS BY THE LIVER OF GUINEA PIGS IN DIFFERENT SEXUAL STATES

THE INACTIVATION of estrogens by the liver and other organs in dogs has been shown previously.[1] Zondek[2] confirmed these results concerning the liver, but denied that any other organ can inactivate estrogens. Heller[3] has recently found that there is an inactivation of the hormone not only by liver but also by the kidneys of rats. In experiments with hepatectomised frogs[4] we were also able to show that in these animals estrone is inactivated, hence, at least in cold blooded animals, the liver is not the only organ which possesses this faculty. Nevertheless there can be no doubt that, except for the endocrines, the liver is the most important organ for the metabolism of estrogens.

Recently Talbot[5] and Pincus and Martin[6] have shown that the activity of estrogens is increased by liver damage. It seemed to us of some interest to study the influence of the sexual state of the animal on the estrogen inactivating potency of the liver.

Livers of guinea pigs were used. In the first report[1] we have shown that more than 80% of estrogens are inactivated in vitro by dog livers in 2 hours at 37° C. as well as in 24 hours at room temperature. In these experiments we allowed the estrogens to remain for 24 hours at room temperature (14° C.) together with liver pulp of the respective animals.

In every experiment we allowed 200 M.U. of estradiol benzoate (0.02 mg. of Dimenformon, Organon) to stand for 24 hours with the pulp of the whole liver of an adult guinea pig. We used the livers of male, female, castrate and pregnant guinea pigs.

After 24 hours the liver pulp was extracted with alcohol, ether, acetone and benzol and the material assayed by the Allen-Doisy test using castrate female mice.

The extracts of the liver pulp with Dimenformon were injected in different quantities to the castrate mice and the loss of estrogenic activity was calculated. The results are given in the following table.

Guinea pig no. and sex	Sexual state	% loss of estrogenic activity by its liver extracts
1 ♂	Normal	66
2 ♂	Castrate	66
3 ♀	Normal	33
4 ♀	Castrate	33
5 ♀	Pregnant	33

The destruction of estrogens by the liver of guinea pigs seems less intense than by dog or rat liver. The inactivating potency of the liver of male animals seems higher than the inactivation by the liver of females. We could not detect a difference in estrogen inactivation depending upon the sexual state of the animal. The liver of a female castrated 6 weeks before showed exactly the same power as the liver of a pregnant guinea pig. It is interesting that there is a difference between male and female livers even in castrate animals.

Summary. The liver of male guinea pigs shows a stronger estrogen inactivating power than the livers of female animals. Influence of the sexual states such as castration or pregnancy upon estrogen inactivating potency of guinea pig liver was not noted.

PAUL ENGEL

Veterinary School
National University
BOGOTÁ, COLOMBIA

[1] SILBERSTEIN, F., P. ENGEL AND K. MOLNAR: *Klin. Wchnschr.* 12: 1693. 1933.
[2] ZONDEK, B.: *Skand. Arch. Physiol.* 70: 133. 1934, Die Hormone des Ovariums und des Hypophysenvorderlappens, 2nd ed. Julius Springer, Vienna, 1936.
[3] HELLER, C. G.: *Endocrinology* 26: 619. 1940.
[4] ENGEL, P., AND E. NAVRATIL: *Biochem. Ztschr.* 292: 434. 1937.
[5] TALBOT, N. B.: *Endocrinology* 25: 601. 1939.
[6] PINCUS, G., AND D. W. MARTIN: *Endocrinology* 26: 838. 1940.